SUMMARY

Particles from all sources collected on the personal monitor front filter are estimated gravimetrically. The ETS contribution to these particles is estimated by UV, fluorescence and solanesol measurements on a methanol extract of the filter.

Nicotine and 3-Ethenylpyridine, collected partly by the front filter and mainly by the second acidic filter, are determined by gas chromatography using a nitrogen selective detector.

Saliva samples taken from the subjects are analysed for cotinine content by gas chromatography with mass selective detection.

2023522556

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

OUTLINE PROCEDURE

ANALYSIS OF THE COLLECTED SAMPLES

DISMANTLE THE HOLDER.

WEIGH THE TEFLON FRONT FILTER TO DETERMINE TSP.

EXTRACT THE TEFLON FILTER WITH METHANOL. ANALYSE FOR UVPM, FPM, SOLANESOL, NICOTINE AND 3-ETHENYLPYRIDINE.

ANALYSE THE ACIDIC FILTER FOR NICOTINE AND 3-ETHENYLPYRIDINE.

ANALYSE THE FILTER HOLDER BLANK FOR THE SAME ANALYTES.

ANALYSE THE SALIVA SAMPLES FOR COTININE.

2023522557

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

WEIGHING AND EXTRACTION OF THE FILTERS PRIOR TO ANALYSIS

EQUIPMENT

1. MICROBALANCE

Mettler M3 (6 decimal place) or equivalent. The balance should be mounted on a very stable surface and situated in a temperature controlled laboratory away from strong draughts.

The microbalance should stand on an earthed antistatic mat. During microweighing, the operator should be connected to this mat via an antistatic wrist-band strap. This arrangement eliminates static charge build-up arising from the operator and ambient conditions during microweighing.

2. CHECK-WEIGHT FOR MICROBALANCE

A 20 mg calibration weight was used as a check weight throughout the study. This weight is approximately the same as the weight of a Millipore filter.

3. RADIOACTIVE SEALED SOURCE STATIC ELIMINATOR

BAR-TYPE:	POLONIUM-210 (approximately 148 M Bcq)
CATALOGUE NUMBER:	PDV 1
SUPPLIER:	Amersham International PLC, Buckinghamshire, England

Polonium-210 radioactive static eliminators have a working lifetime of approximately one year.

4. GLASSWARE

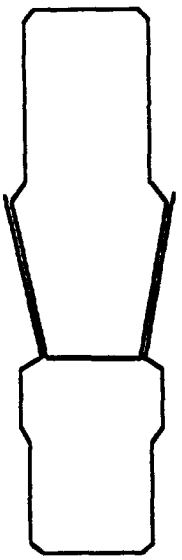
Extraction pots, with ground glass stoppers (See Appendix 4 Figure 1).

Test tubes, 15 mL, with screw top fitting and screw caps with Teflon inner lining.

2023522558

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

APPENDIX 4 FIGURE 1



34/35 GROUND GLASS STOPPER

GROUND GLASS JOINT

27 mm ID VESSEL TO ACCOMMODATE FILTER PARTS

EXTRACTION POT

2023522559

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

REAGENTS

Methanol: HPLC grade, 99.9% minimum (Romil Super Purity or equivalent)

Di-isopropyl ether (DIPE): HPLC grade, 99.9% minimum (Romil Super Purity or equivalent)

DIPE solvent. Containing (1) 0.1 mL/litre triethylamine

(2) 2.0 mg/litre N-ethylnornicotine.

Solanesol: (Sigma), 90%+. Purity checked against purified solanesol (98%+)

Nicotine: Double distilled 99%+

3 Ethenylpyridine: Double distilled 98%+ (containing 0.5% hydroquinone as a stabiliser)

N-Ethylnornicotine: Double distilled 99%+

Cotinine: (Lancaster Synthesis) 98%

N-Ethylnorcotinine 98%

Scopoletin: (Aldrich) 95%

2,2',4,4'-Tetrahydroxybenzophenone: (Aldrich) 97%

Triethylamine: (BDH) 99.5%

Dichloromethane: (BDH) GC analysis grade

2023522560

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

DISMANTLE THE HOLDER

Break the security tag and unscrew the clamping cover (see Appendix 4 Figure 2).

Carefully remove the filter holder entrance plate while keeping the holder upright to ensure that the filters stay in place.

WEIGH THE TEFLON FRONT FILTER TO DETERMINE TSP

Carefully remove the Millipore filter from the holder with clean stainless steel tweezers.

Ensure the balance has gone through its daily calibration check and that the check-weight is within 1 μg of the last check and 2 μg of the expected value determined at the start of the project.

Weigh the filter by the same procedure as used when the filter was weighed before the sampling period.

Calculate the weight of particulate matter collected on the filter from the difference in weight before sampling and after sampling.

Calculate the weight of particulate matter in μg collected per cubic metre.

TSP=(W2-W1)*1000/VA μg/m3

Where:

W1=weight of filter before sampling in μg

W2=weight of filter after sampling in μg

VA=volume of air sample collected in litres

EXTRACT THE TEFLON FRONT FILTER AND TEFLON SEAL WITH METHANOL FOR SUBSEQUENT ANALYSIS FOR UVPM, FPM, SOLANESOL, NICOTINE AND 3-ETHENYLPYRIDINE

2023522561

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

EXTRACT THE ACID FILTER FOR NICOTINE AND 3-ETHENYLPYRIDINE ANALYSIS

Remove the first Teflon spacer, the acid-treated Pallflex filter and the second Teflon spacer from the filter holder using clean stainless steel tweezers. Transfer these to an extraction pot and add 1.0 mL DIPE (containing internal standard and triethylamine) followed by 4 mL 5N sodium hydroxide. Stopper the extraction pot and shake gently for 30 minutes.

Transfer as much of the liquid as possible to a screw topped 15 mL test tube and cap the tube with a Teflon lined cap.

Allow the phases to separate and then transfer about 0.5 mL of the upper (DIPE) layer to an autosampler vial and cap the vial. Take care not to transfer any of the aqueous phase.

This vial will be used for measurement of nicotine and 3-Ethenylpyridine collected on the second filter. Store the vial in the freezer (-20°C) until ready for use. (Referred to as Vial C).

Place the Millipore filter, the Teflon seal, the metal 'O' ring and the metal grid (See Figure 2) in a clean extraction pot and seal with a glass stopper.

Add 5.0 mL of methanol. Stopper the extraction pot and shake gently for 30 minutes. Pipette 1.0 mL of the methanol into a screw topped 15 mL test tube. Add 1.0 mL of DIPE (containing internal standard and triethylamine) and 4.0 mL of 5N sodium hydroxide. Cap the tube with a Teflon lined cap. Mix thoroughly for 10 minutes using a vortex mixer. Allow the phases to separate and then transfer at least 0.5 mL of the upper (DIPE) layer to an autosampler vial and cap the vial. Take care not to transfer any of the aqueous phase.

This vial will be used for measurement of any nicotine and 3-Ethenylpyridine collected on the first filter. Store the vial in the freezer (-20°C) until ready for use. (Referred to as Vial A).

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Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Fill a 2 mL autosampler vial with some of the remaining methanol extract and cap the vial. This vial will be used for measurement of UVPM, FPM and solanesol. Store the vial in the freezer (-20°C) until ready for use. (Referred to as Vial B).

Transfer the remaining methanol extract to an autosampler vial for backup purposes. Store this vial in a freezer (-20°C) until required for use.

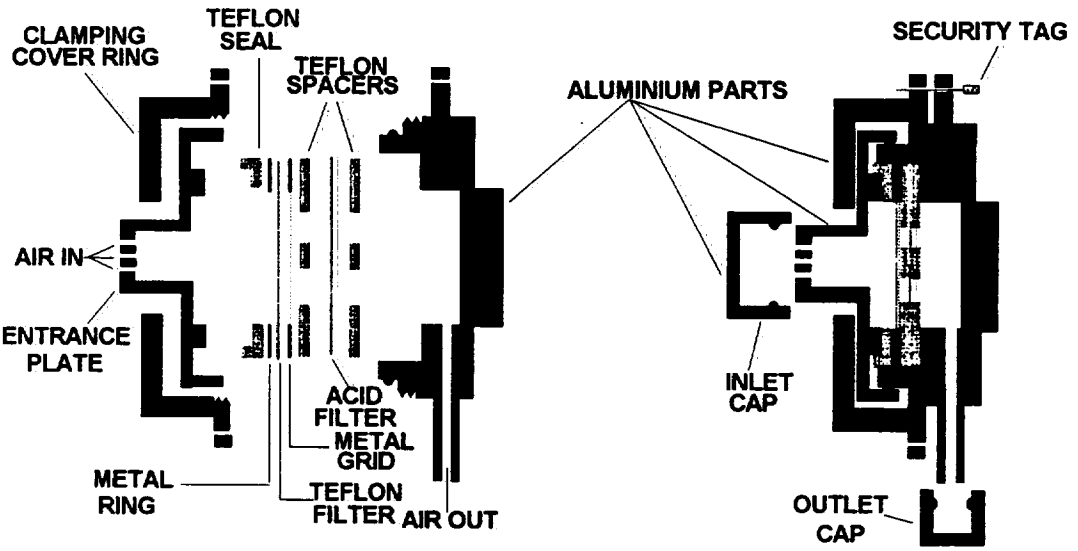
ANALYSE THE FILTER HOLDER BLANK FOR THE SAME ANALYTES

Dismantle the blank filter holder and carry out the same weighing and extractions as for the sample filter holders.

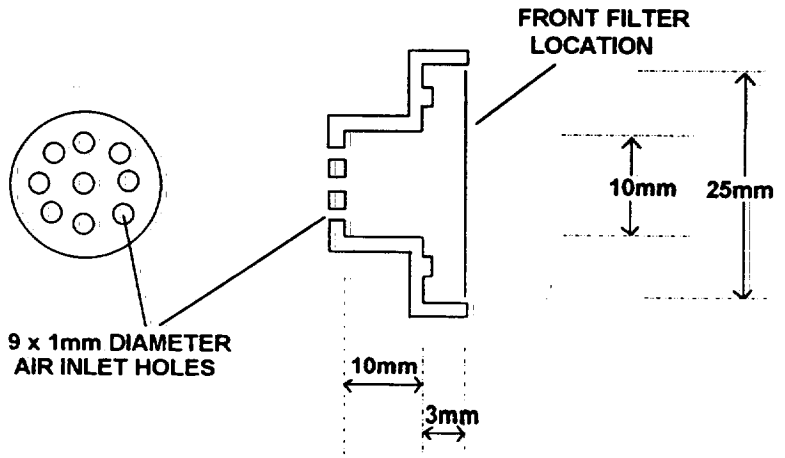
2023522563

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

APPENDIX 4 FIGURE 2



FILTER HOLDER APART AND ASSEMBLED



ENTRANCE PLATE DIMENSIONS

2023522564

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

ESTIMATION OF UVPM AND FPM

PRINCIPLE

Particulate matter collected on a Millipore Teflon filter is extracted with methanol. UV and fluorescence measurements are made on this extract and these are compared to calibrations made using surrogate standards for ETS particles.

The calibration of surrogate standards against ETS has been established in prior experiments using ETS generated in a Model Room using the same master calibration solutions. This enables the UV and Fluorescence measurements to be converted to a weight of ETS particles.

EQUIPMENT

HPLC Pump capable of pumping methanol at 1.0 mL/min.

UV detector set at 325 nm, cell volume 14 μL, path length 10 mm.

Fluorescence detector with Excitation 300 nm and Emission 420 nm, cell volume 16 μL, path length 10 mm.

Thermostatted column oven set at 30°C

Delay tube / restrictor: Empty stainless steel tube, 4 m long, 0.33 mm ID

Frit filter

Loop injector with 50 μL loop.

The equipment is set up as a column-less HPLC system with the fluorescence detector and UV and detector used in series.

REAGENTS

Methanol: HPLC grade, 99.9% minimum (Romil Super Purity or equivalent)

Scopoletin: (Aldrich) 95%

2,2',4,4'-Tetrahydroxybenzophenone: (Aldrich) 97%

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Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

CALIBRATION

1. UVPM

Prepare (by dilution from a stronger solution) a master solution containing 12.5 mg of 2,2',4,4'-Tetrahydroxybenzophenone (THBP) in 2.5 litres of methanol (ie 5 μg/mL).

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with methanol as follows:

CONCENTRATION μg/mL	DILUTION OF MASTER	EQUIVALENT UVPM μg/mL
2.5	50 mL to 100 mL	15.25
1.0	20 mL to 100 mL	6.1
0.5	10 mL to 100 mL	3.05
0.25	5 mL to 100 mL	1.53
0.10	2 mL to 100 mL	0.61
0.05	1 mL to 100 mL	0.31
0.00	0 mL to 100 mL	0

These calibration solutions cover a range equivalent to 0 - 380 μg/m^3 of ETS particles. The 0.05 μg/mL calibration point corresponds to 7.6 μg/m^3 ETS particles.

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

2. FPM

Prepare (by dilution from a stronger solution) a master solution containing 2.5 mg scopoletin in 2.5 litres of methanol (ie 1 μg/mL).

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Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with methanol as follows:

CONCENTRATION μg/mL	DILUTION OF MASTER	EQUIVALENT UVPM μg/mL
0.50	50 mL to 100 mL	11.25
0.25	25 mL to 100 mL	5.63
0.10	10 mL to 100 mL	2.25
0.050	5 mL to 100 mL	1.13
0.020	2 mL to 100 mL	0.45
0.010	1 mL to 100 mL	0.23
0.00	0 mL to 100 mL	0

These calibration solutions cover a range equivalent to 0 to 281 $\mu g/m^3$ of ETS particles. The calibration point 0.01 μg/mL corresponds to 5.6 $\mu g/m^3$ ETS particles.

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

Fill autosampler vials with each of the THBP and scopoletin calibration solutions.

Make duplicate injections of each calibration solution into the combined UVPM/FPM system using the loop injector. Measure the peak area of the UV absorbance peak using an integrator.

Prepare a calibration graph of UV Absorbance Peak Area against UVPM concentration (as calculated from the THBP concentration).

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Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Prepare a calibration graph of Fluorescence Emission Peak Area against FPM Concentration (as calculated from the scopoletin concentration).

METHOD

Make duplicate injections of the methanol extract of the particulate matter (collected on the Millipore Teflon filter) from its autosampler vial (Referred to as Vial B) into the UVPM/FPM system using the loop injector.

Determine the UV Absorbance Peak Area and the Fluorescence Emission Peak Area.

CALCULATION

By reference to the calibration graphs (or the equation of best fit), determine the UVPM concentration and FPM concentration of the sample solution in μg/mL.

Calculate the quantities of UVPM and FPM per cubic metre of air sampled as follows:

UVPM=U*VM*1000/VA μg/m3

FPM=F*VM*1000/VA μg/m3

Where:

U=UVPM concentration in methanol extract, in μg/mL

F=FPM concentration in methanol extract, in μg/mL

VM=volume of methanol used to extract the Millipore filter, in mL

VA=volume of air sample collected, in litres

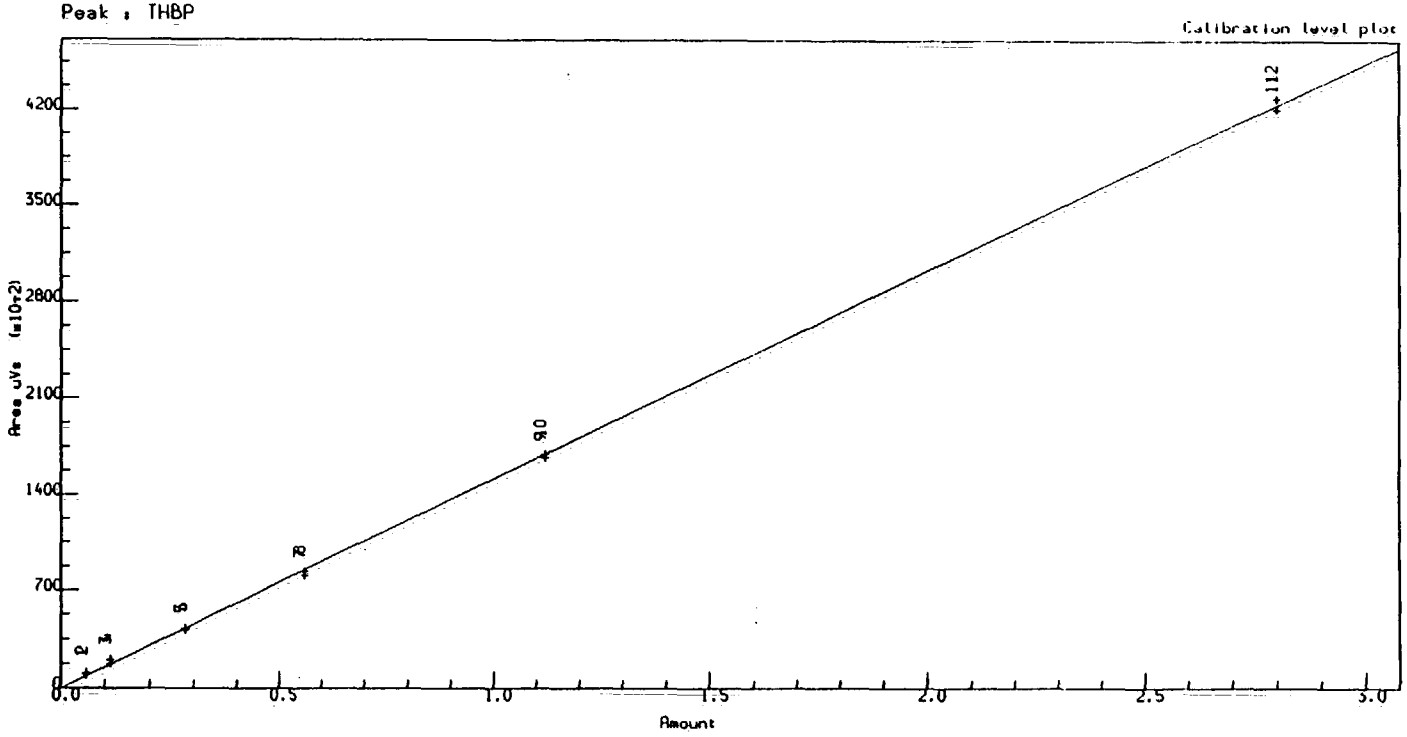
Correct the results for values obtained from the blank.

2023522568

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Calibration Name : 3 UV28-10.
UV28-10
Peak : THBP



Constant : -3.966904E+1
1st degree : 1.51488E+5

Curve fit : Linear
Correlation coefficient : 0.99984
Standard error : 2.83721E+3

Reported on 26-NOV-1992 at 14:09 R.

JM 26/11/92

Typical UVPM calibration plot
(using THBP standard)

HUK Study no 12/64

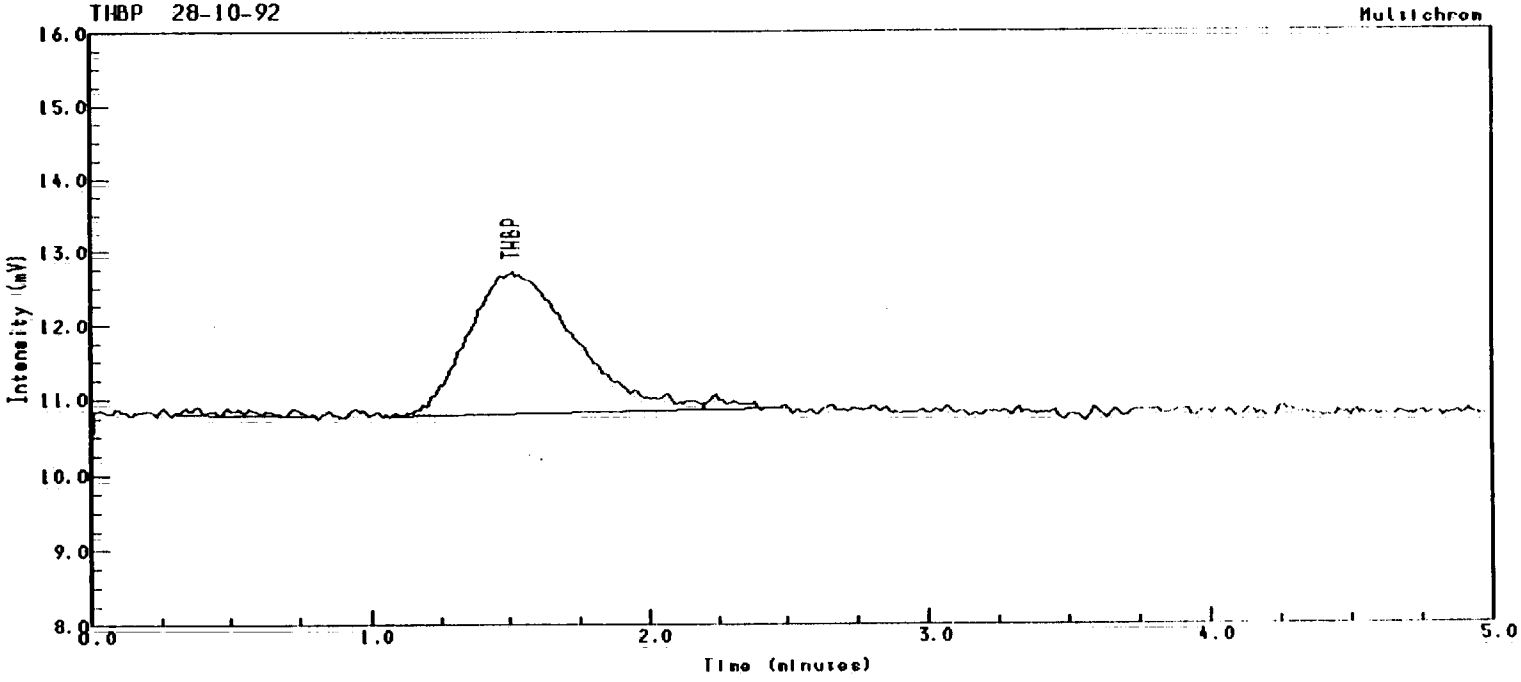
69522352023

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical sample UV absorbance for UVPM

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 3 UV28-10.28.1.
28-10/118-06 Amount : 1.000
THBP 28-10-92



Instrument :
Channel Title : Channel #3
Lims ID :
Acquired on 30-OCT-1992 at 21:51
Reported on 26-NOV-1992 at 14:14

Method : UV28-10
Calibration : UV28-10
Run Sequence : UV28-10

JM

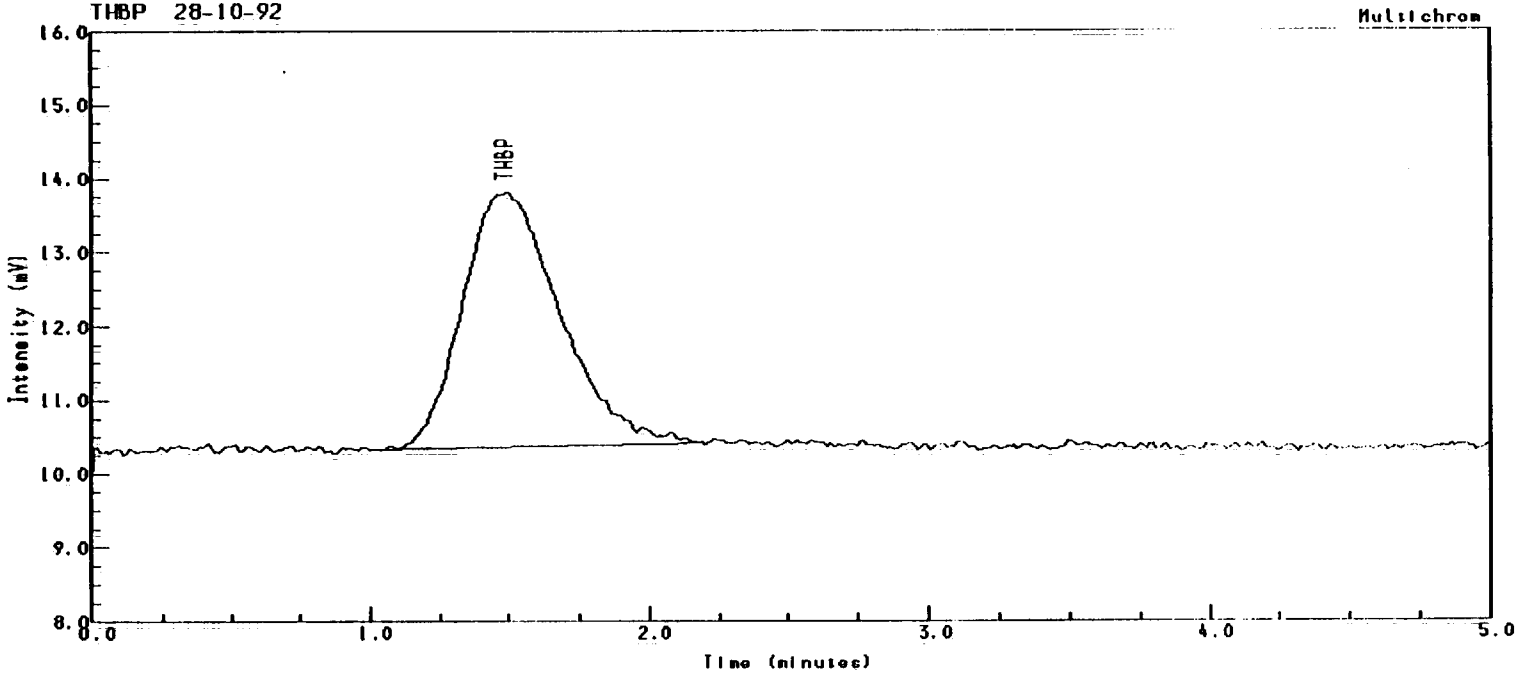
02352257O

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard UV absorbance for UVPM
(0.5 µg/mL THBP)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 3 UV28-10.22.1.
UCal 0.50 Amount : 1.000
THBP 28-10-92



Instrument :
Channel Title : Channel #3
Lims ID :
Acquired on 30-OCT-1992 at 21:01
Reported on 26-NOV-1992 at 14:14

Method : UV28-10
Calibration : UV28-10
Run Sequence : UV28-10

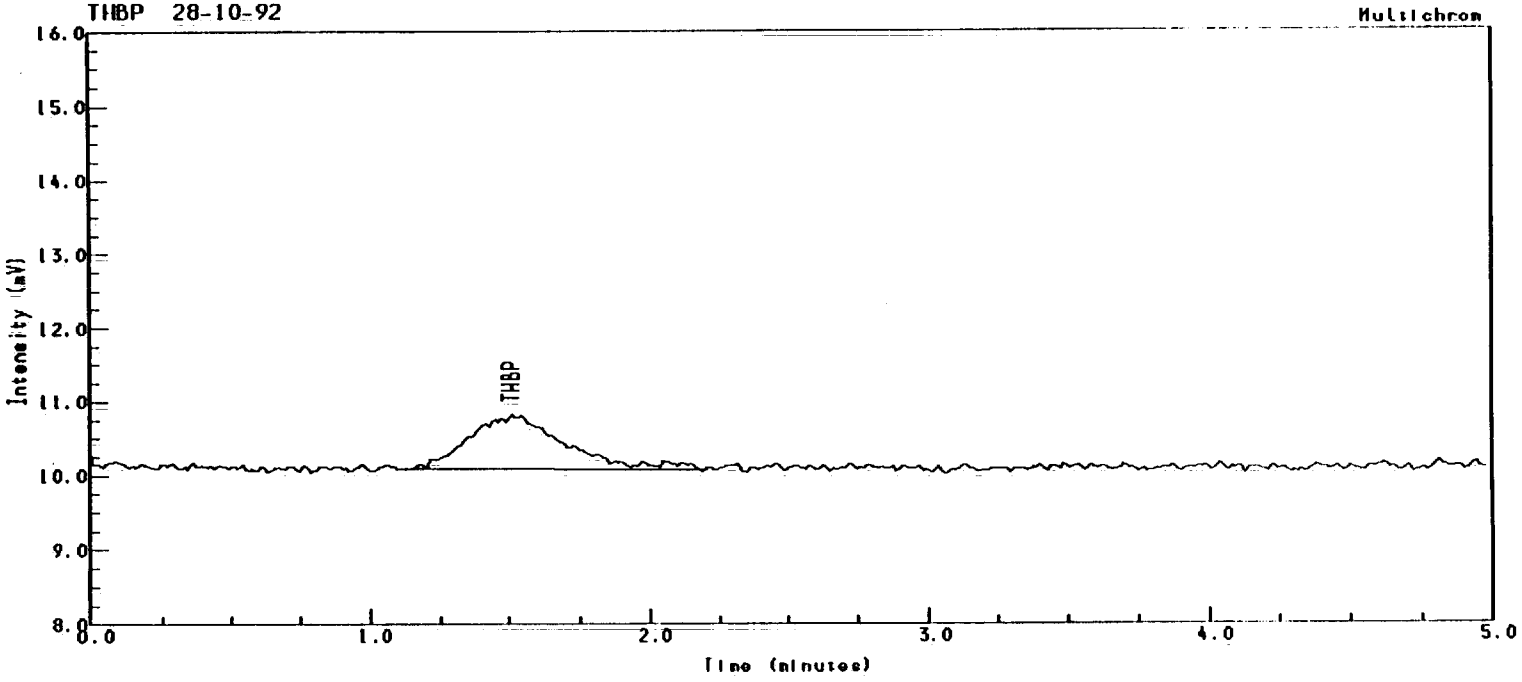
JM

20235225721

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 3 UV28-10,17.1.
UCal 0.10 Amount : 1.000
THBP 28-10-92



Instrument :
Channel Title : Channel #3
Lims ID :
Acquired on 30-OCT-1992 at 20:21
Reported on 26-NOV-1992 at 14:13

Method : UV28-10
Calibration : UV28-10
Run Sequence : UV28-10

JM

Typical standard UV absorbance for UVPM
(0.1 µg/mL THBP)

2023522572

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

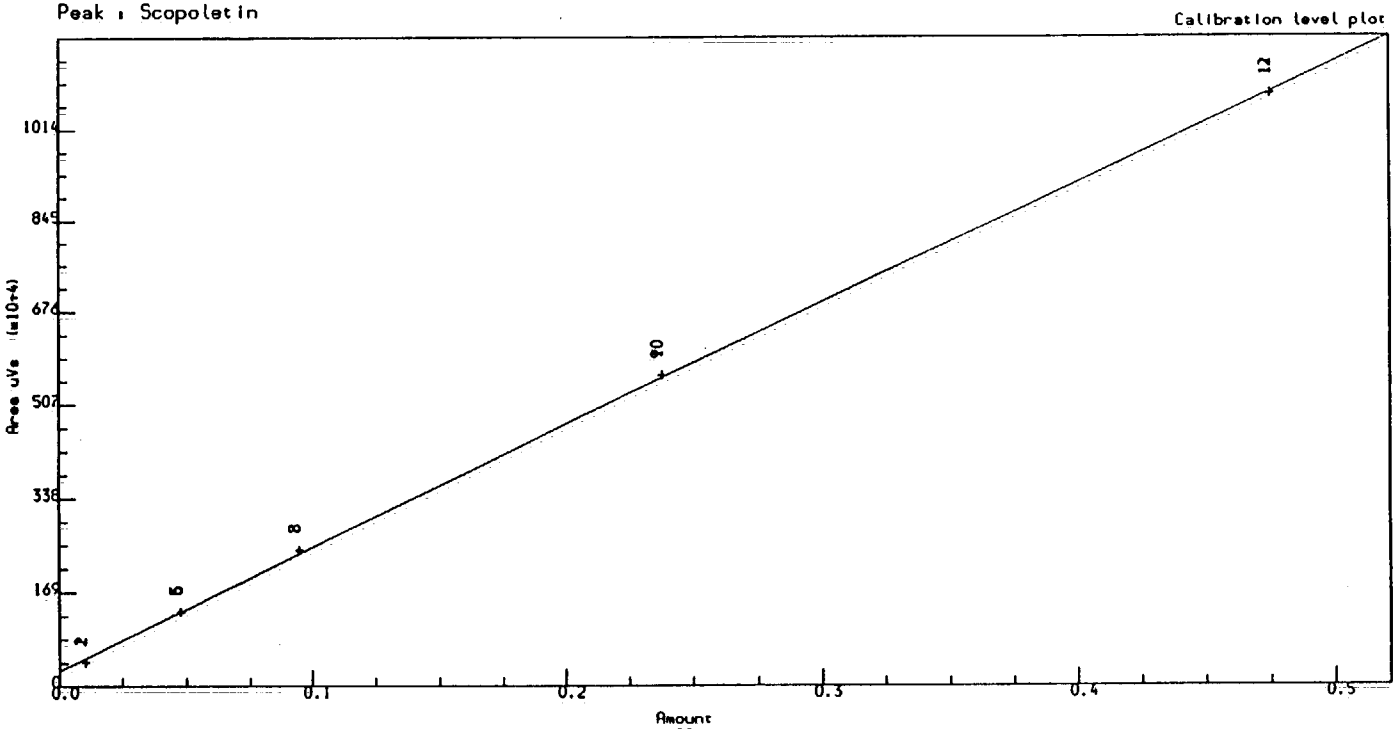
Typical FPM calibration plot
(using Scopoletin as standard)

HAZLETON Bioanalytical department Multichrom 1.8

Calibration Name : 1 F5-11.

F5-11

Peak : Scopoletin



Constant : 2.81482E+5
1st degree : 2.21498E+7

RU

JHP 26-11-92

Curve fit : Linear
Correlation coefficient : 0.99992
Standard error : 5.44278E+4

Reported on 23-NOV-1992 at 15:24

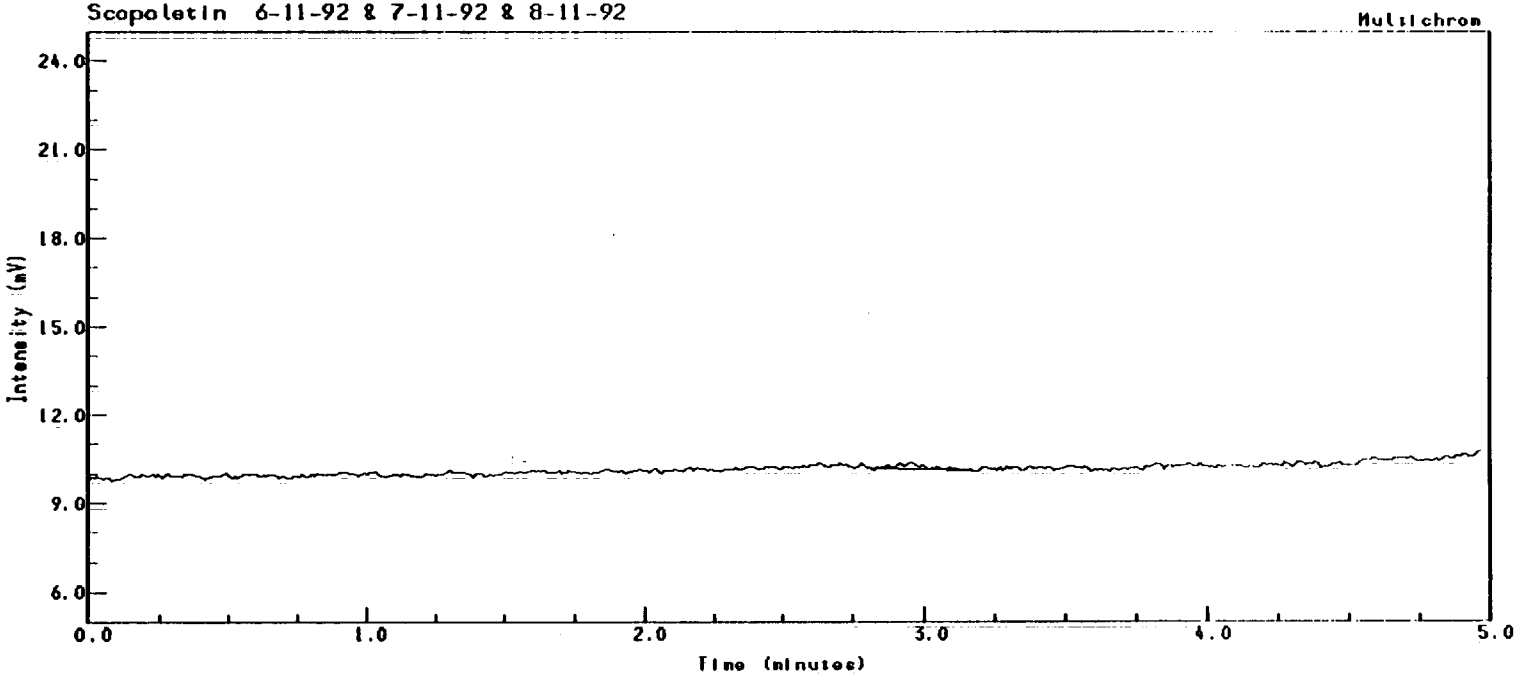
20235225273

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration fluorescence response (blank)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 1 F5-11.1.1.
Cal 0 (MeOH) Amount : 1.000
Scopoletin 6-11-92 & 7-11-92 & 8-11-92



Instrument :
Channel Title : Channel 1
Lims ID :
Acquired on 10-NOV-1992 at 20:53
Reported on 23-NOV-1992 at 15:48

Method : F5-11
Calibration : F5-11
Run Sequence : F5-11

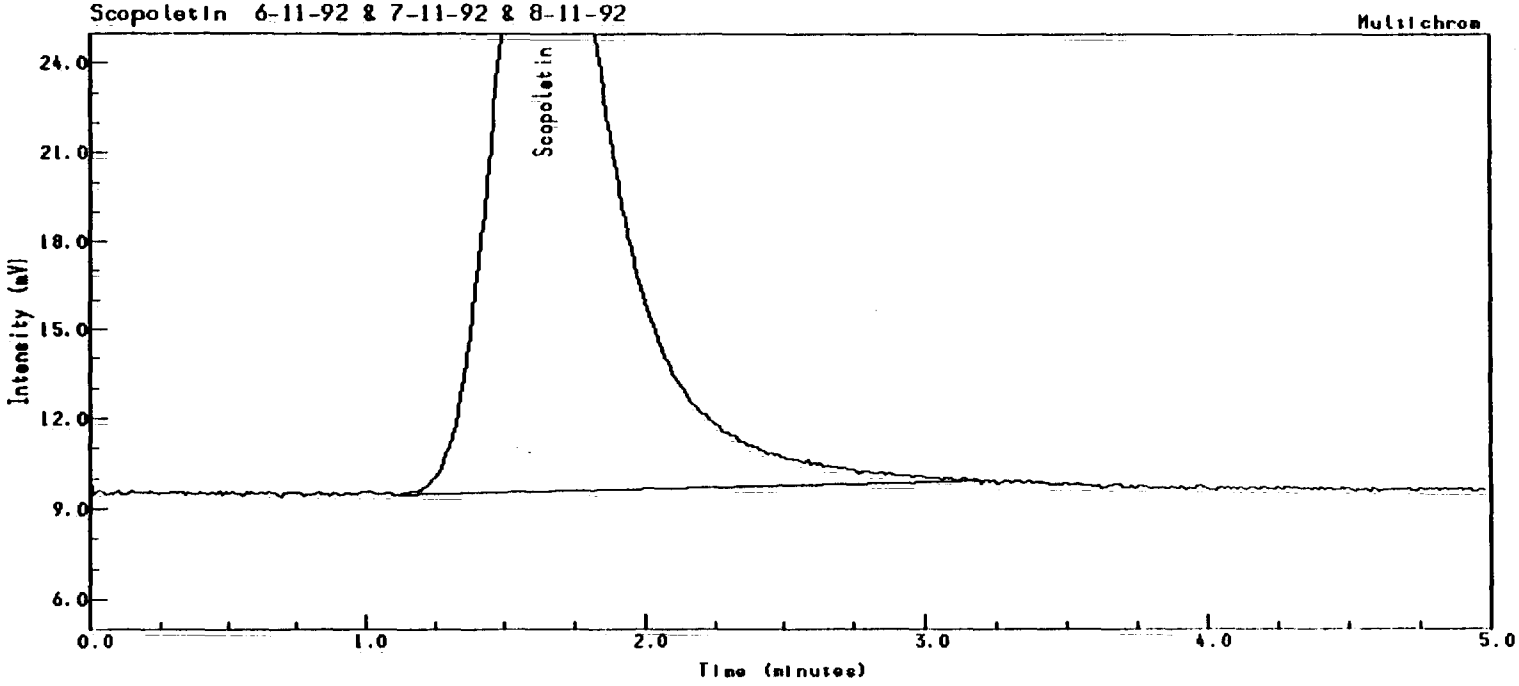
20235225574

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical sample fluorescence response

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 1 F5-11.44.1.
07-11/169-14 Amount : 1.000
Scopoletin 6-11-92 & 7-11-92 & 8-11-92



Instrument :
Channel Title : Channel 1
Lims ID :
Acquired on 11-NOV-1992 at 02:42
Reported on 23-NOV-1992 at 15:55

Method : F5-11
Calibration : F5-11
Run Sequence : F5-11

Q.C

SHP

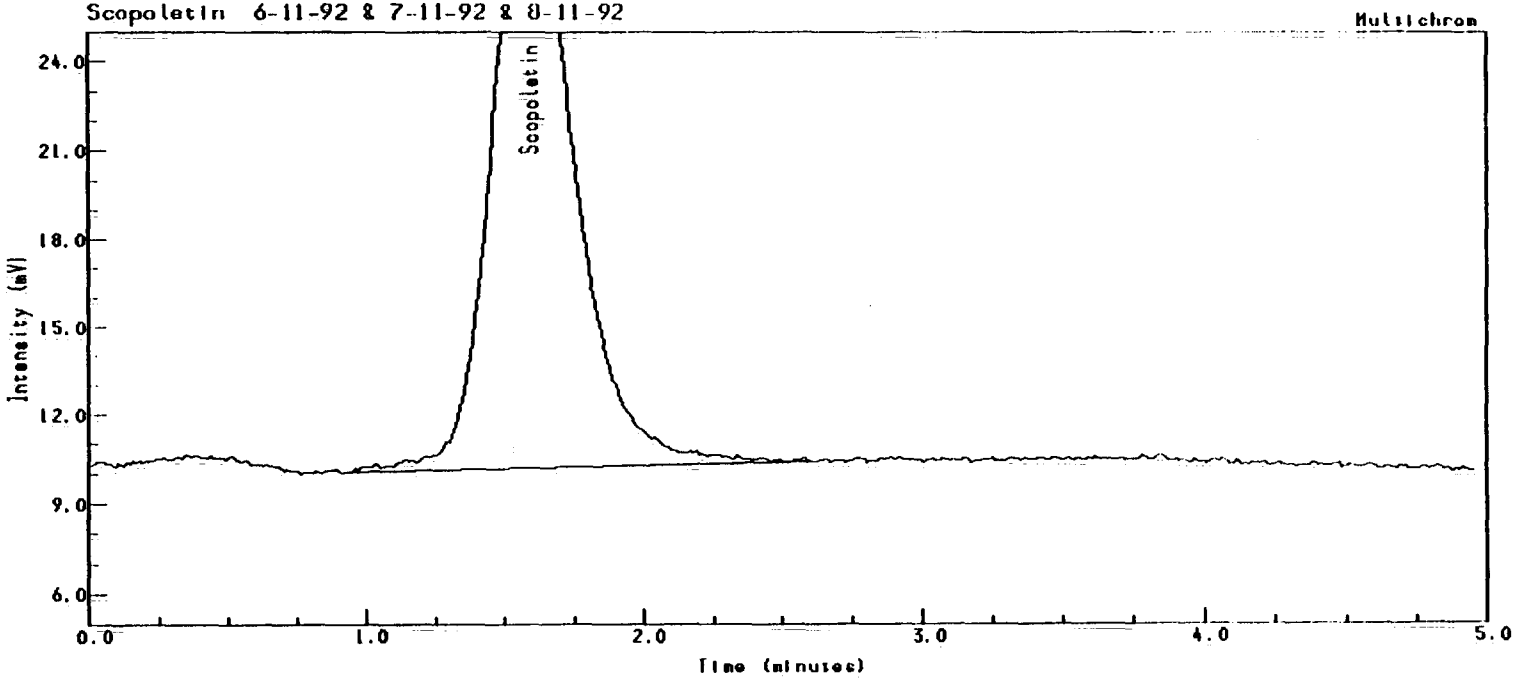
2023225275

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration fluorescence response (0.01 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 1 F5-11,3,1.
FCal 0.01 Amount : 1.000
Scopoletin 6-11-92 & 7-11-92 & 8-11-92



Instrument :
Channel Title : Channel 1
Lims ID :
Acquired on 10-NOV-1992 at 21:10
Reported on 23-NOV-1992 at 15:48

Method : F5-11
Calibration : F5-11
Run Sequence : F5-11

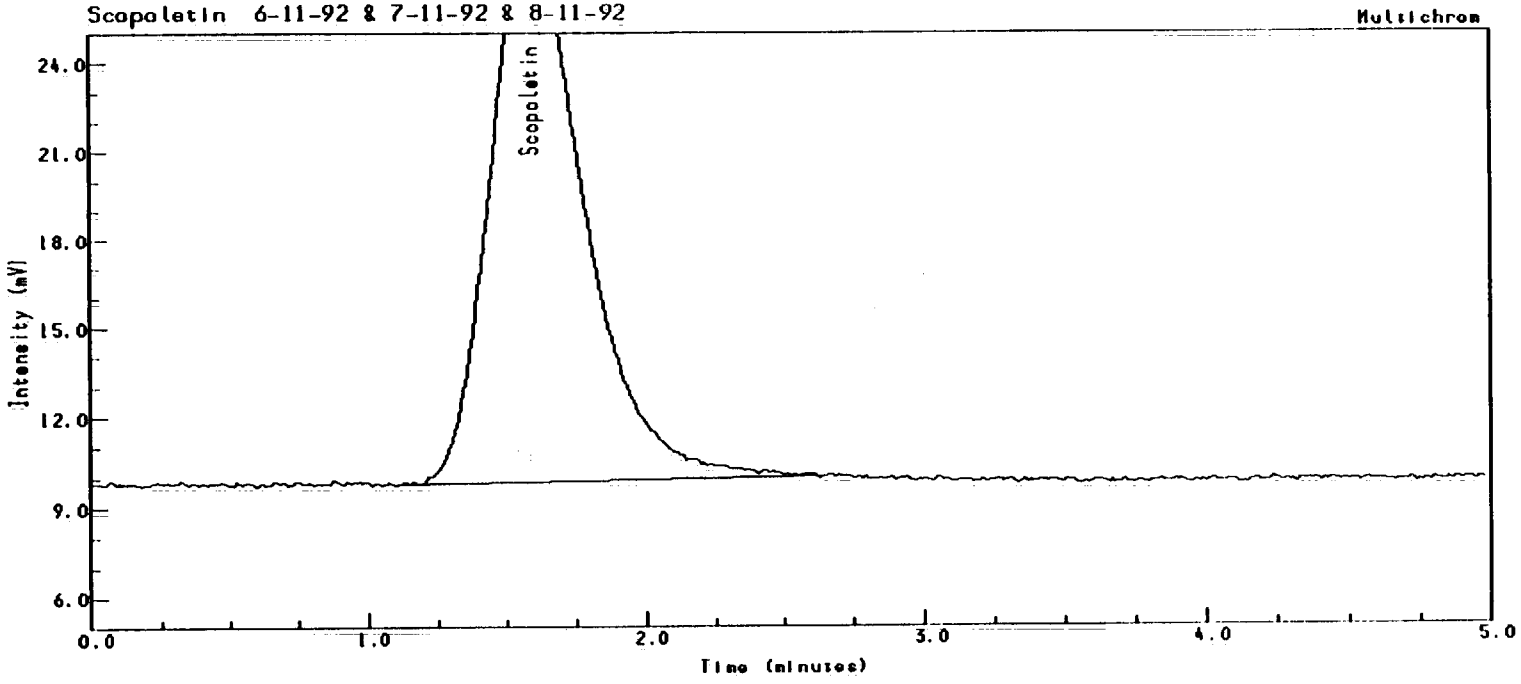
2023522576

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration fluorescence response (0.02 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 1 FS-11,6,1.
FCal 0.02 Amount : 1.000
Scopoletin 6-11-92 & 7-11-92 & 8-11-92



Instrument :
Channel Title : Channel 1
Lims ID :
Acquired on 10-NOV-1992 at 21:35
Reported on 23-NOV-1992 at 15:49 R.J

Method : FS-11
Calibration : FS-11
Run Sequence : FS-11

FAP.

2023522577

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

DETERMINATION OF SOLANESOL CONTENT AND SOLANESOL-BASED ETS PARTICLES (SPM)

PRINCIPLE

Particulate matter collected on a Millipore Teflon filter is extracted with methanol.

The Solanesol content of this extract is measured by HPLC.

Solanesol-based ETS particulate matter is calculated using a pre-determined factor which is calculated from the solanesol content of ETS particles generated in a Model Room.

EQUIPMENT

HPLC Pump capable of pumping methanol at 2.0 mL/min.

UV detector set at 210 nm, cell volume 8 μL, path length 10 mm.

Thermostatted column oven set at 30°C

Guard column:(Waters) Guard-Pak C18

Analytical column: Such as 15 cm (or 25 cm) Spherisorb ODS 2, 5 micron

Loop injector with 200 μL loop.

REAGENTS

Methanol, HPLC grade, 99.9% minimum(Romil Super Purity or equivalent)

Solanesol: (Sigma), (90%+) further purified by sponsor to 98%+.

CALIBRATION

Prepare (by dilution from a stronger solution) a master solution containing 2.5 mg of solanesol in 2.5 litres of methanol (ie 1 μg/mL).

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

2023522578

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Prepare calibration solutions by dilution of the master solution with methanol as follows:

CONCENTRATION µg/mL	DILUTION OF MASTER	EQUIVALENT UVPM µg/mL
0.5	50 mL to 100 mL	19.25
0.25	25 mL to 100 mL	9.63
0.10	10 mL to 100 mL	3.85
0.050	5 mL to 100 mL	1.93
0.020	2 mL to 100 mL	0.77
0.010	1 mL to 100 mL	0.39
0.005	50 mL 0.01 µg/mL to 100 mL	0.19
0.00	0 mL to 100 mL	0

These calibration solutions cover a range of 0 to 480 $\mu g/m^3$ of SPM. The calibration point of 0.005 µg/mL corresponds to 4.8 $\mu g/m^3$.

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

Fill an autosampler vial with each of the solanesol calibration solutions.

Make duplicate injections of each calibration solution into the HPLC system using the loop injector.

Allow the separation to continue to 1.5 times the solanesol retention time in order to elute later peaks.

Measure the peak area of the UV absorbance peak corresponding to solanesol using an integrator. For the lower level calibrations manual peak height measurements are more reliable than integrator peak areas.

Prepare a calibration graph of Peak Area against solanesol concentration.

2023522579

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

TAKE SALIVA SAMPLE

Remind the subject that a saliva sample is required at the beginning and end of the sampling period. Reassure the subject if necessary that only a chemical test and no medical tests will be done on the sample.

Collect the saliva sample according to the procedure in Figures 3, A and B.

Check that the sample tube is correctly labelled with the subject's code number and the date. Also ensure that the tube is labelled "pre-sample".

Centrifuge the salivette and transfer to a freezer (-20°C) as soon as possible and retain there until ready for use.

CONNECT THE PUMP AND RECORD THE PUMP COUNTER READING

Fit the pump into the belt bag with the plastic tubing passing through the hole provided in the bag.

Remove the filter holder outlet cap and connect the plastic tubing from the pump to the filter holder.

Record the pump stroke counter reading.

REMOVE CAP FROM FILTER HOLDER

Remove the filter holder cap from the air inlet. Do not leave the caps with the subject.

FIT THE MONITOR, START THE PUMP AND RECORD THE PUMP START TIME

Attach the 'necklace' to the filter holder and fit the personal monitor to the subject. Safety pins can be used in place of, or in addition to the necklace if necessary for a particular subject.

Switch on the pump and ensure that it runs at normal speed for at least 30 seconds. Record the pump start time.

2023522580

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

METHOD

Make duplicate injections of the methanol extract of the particulate matter (collected on the Millipore Teflon filter) from its autosampler vial (referred to as vial B) into the HPLC system using the loop injector.

Determine the Peak Area/Peak Height corresponding to solanesol using an integrator/ruler.

CALCULATION

By reference to the calibration graph (or the equation of best fit), determine the solanesol concentration of the sample solution in μg/mL.

Calculate the quantity of solanesol per cubic metre of air sampled as follows:

Solanesol=S*VM*1000/VA μg/m3

Calculate the quantity of SPM per cubic metre of air sampled as follows:

SPM=Solanesol*FS μg/m3

Where:

S=solanesol concentration in methanol extract, in μg/mL

VM=volume of methanol used to extract the Millipore filter, in mL

VA=volume of air sample collected, in litres.

FS=Factor to convert solanesol to SPM

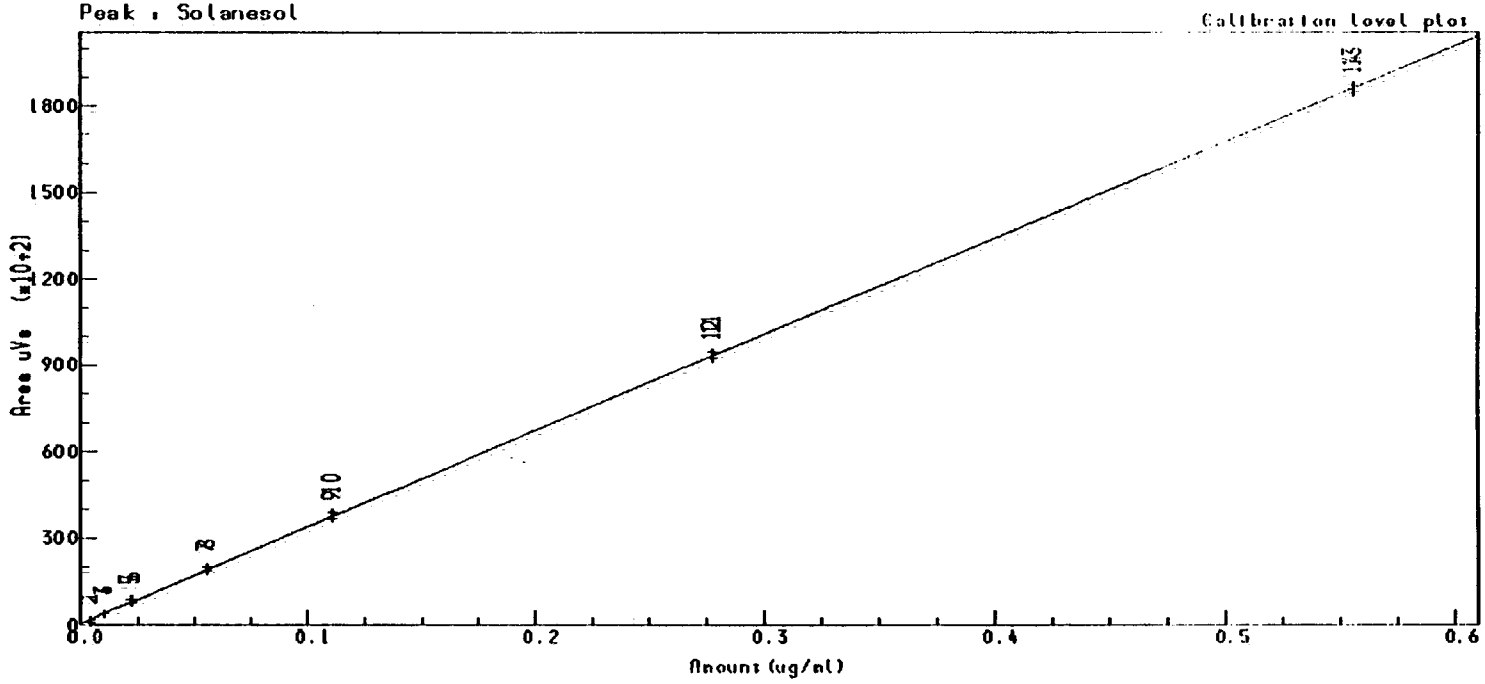
Correct the results for values obtained from the blank.

2023522581

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Calibration Name : 4 S1-11.
S1-11
Peak : Solanesol



Constant : 4.89181E+2
1st degree : 3.34367E+5

Curve fit : Linear
Correlation coefficient : 0.99988
Standard error : 1.03140E+3

Reported on 3-DEC-1992 at 13:09

R.V.

Solanesol calibration plot

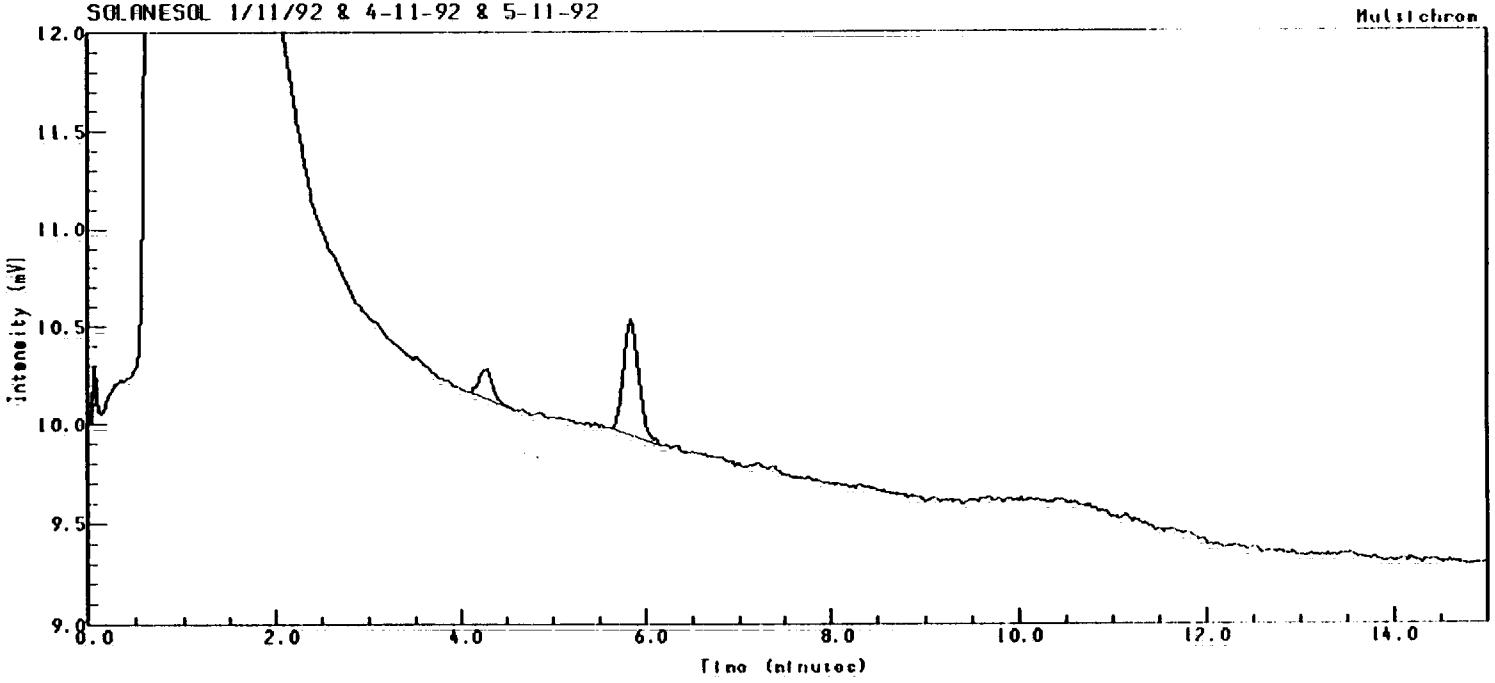
HUK Study no 12/64

28522523202

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11,20,1.
01-11/141-15 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 9-NOV-1992 at 00:17
Reported on 3-DEC-1992 at 13:19

Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

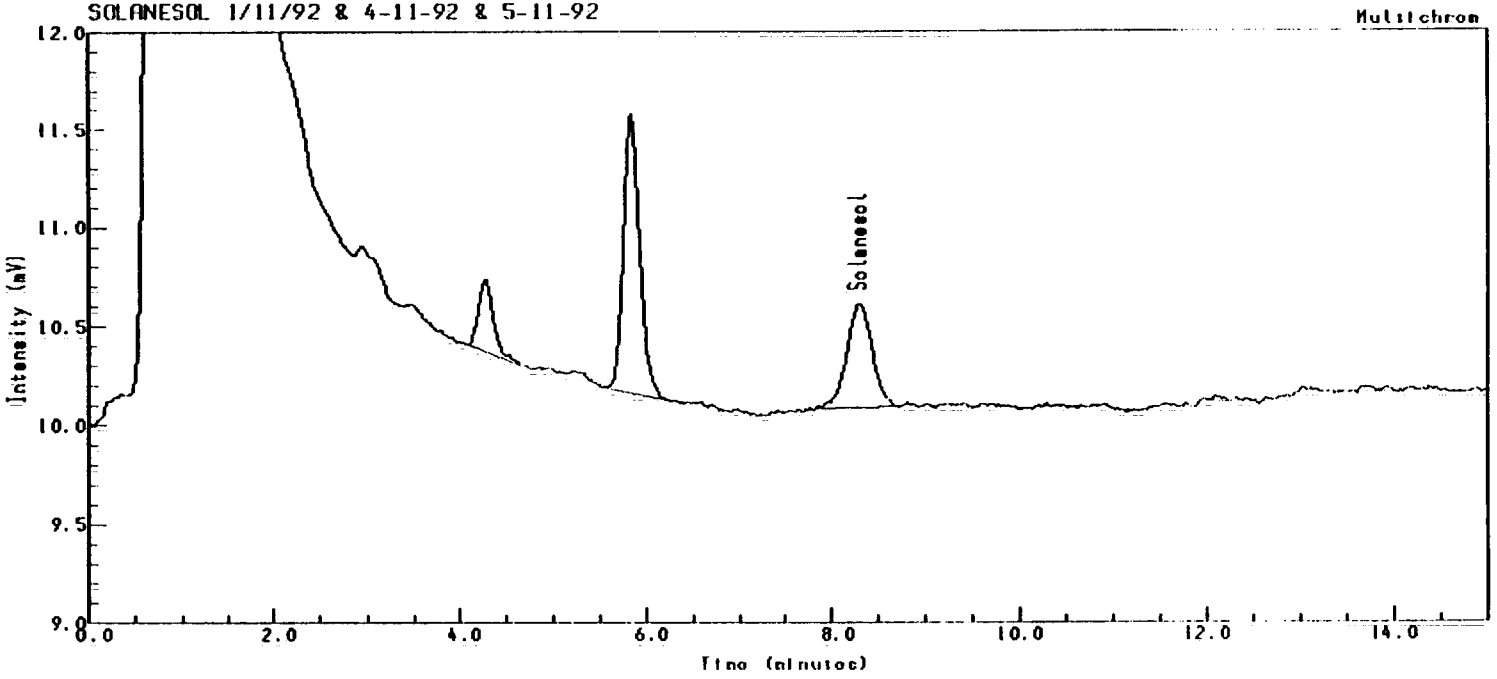
Typical sample chromatogram
(solanesol absent)

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical sample chromatogram
(solanesol present)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11,24,1.
01-11/143-19 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 9-NOV-1992 at 01:32
Reported on 3-DEC-1992 at 13:19

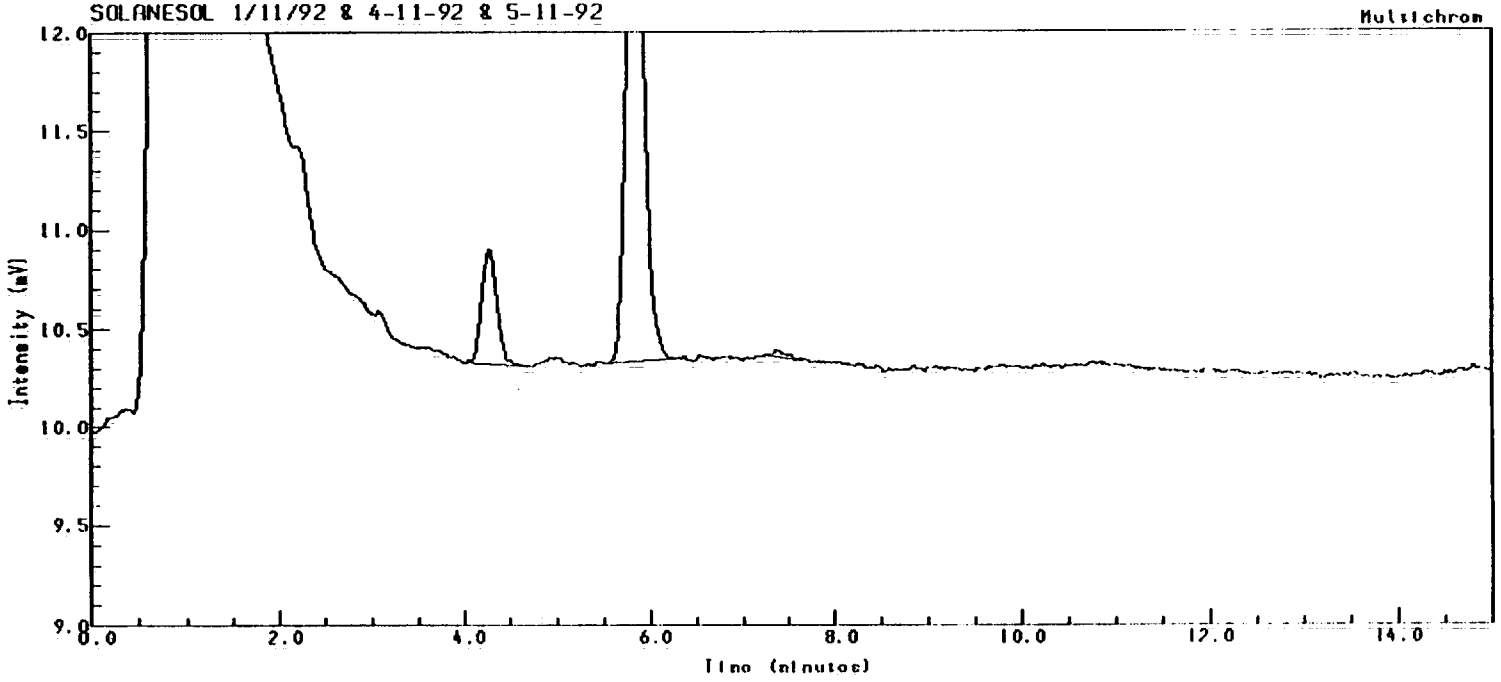
Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

2023225284

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11,28,1.
01-11/BLK-21 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 9-NOV-1992 at 02:49
Reported on 3-DEC-1992 at 13:20

Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

Typical control blank

HUK Study no 12/64

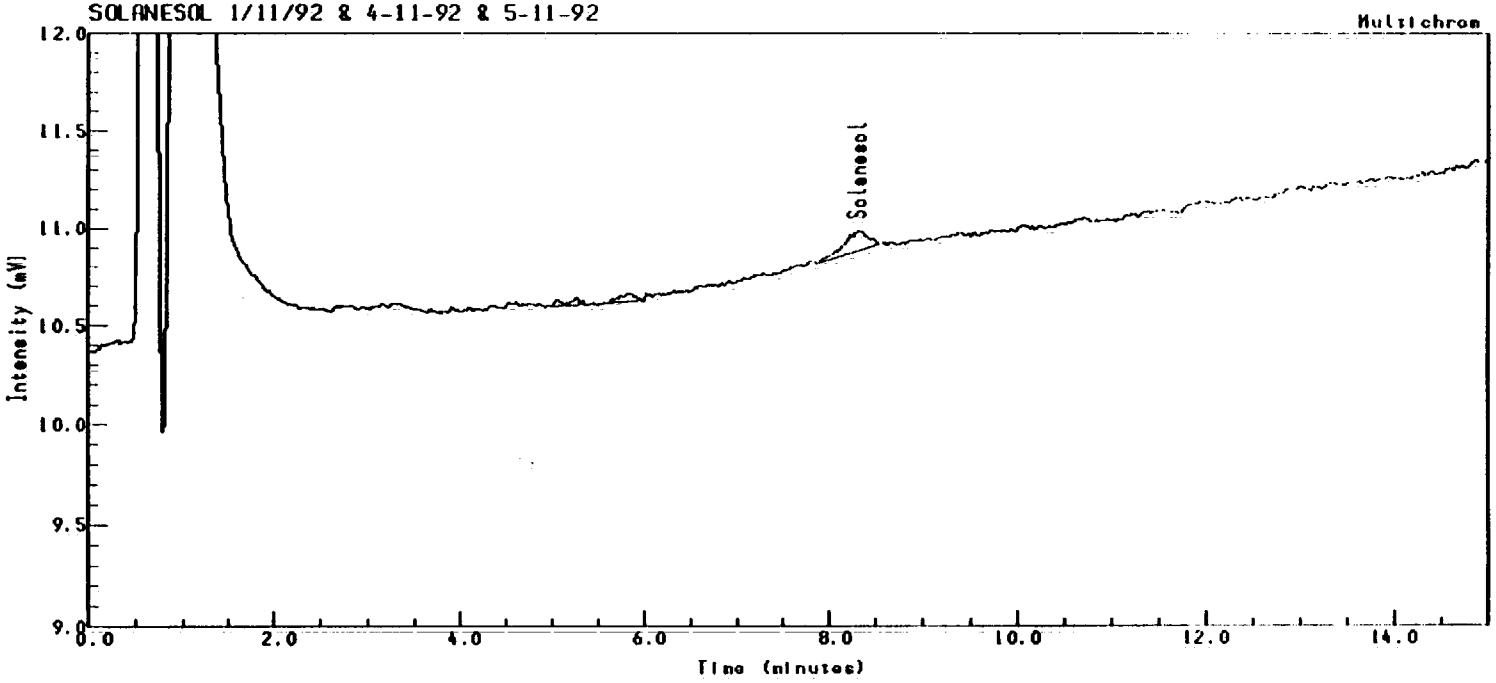
2023252585

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration chromatogram
(0.05 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11,3,1.
Cal 0.005 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 8-NOV-1992 at 18:56
Reported on 3-DEC-1992 at 13:16

Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

JM 2.0.

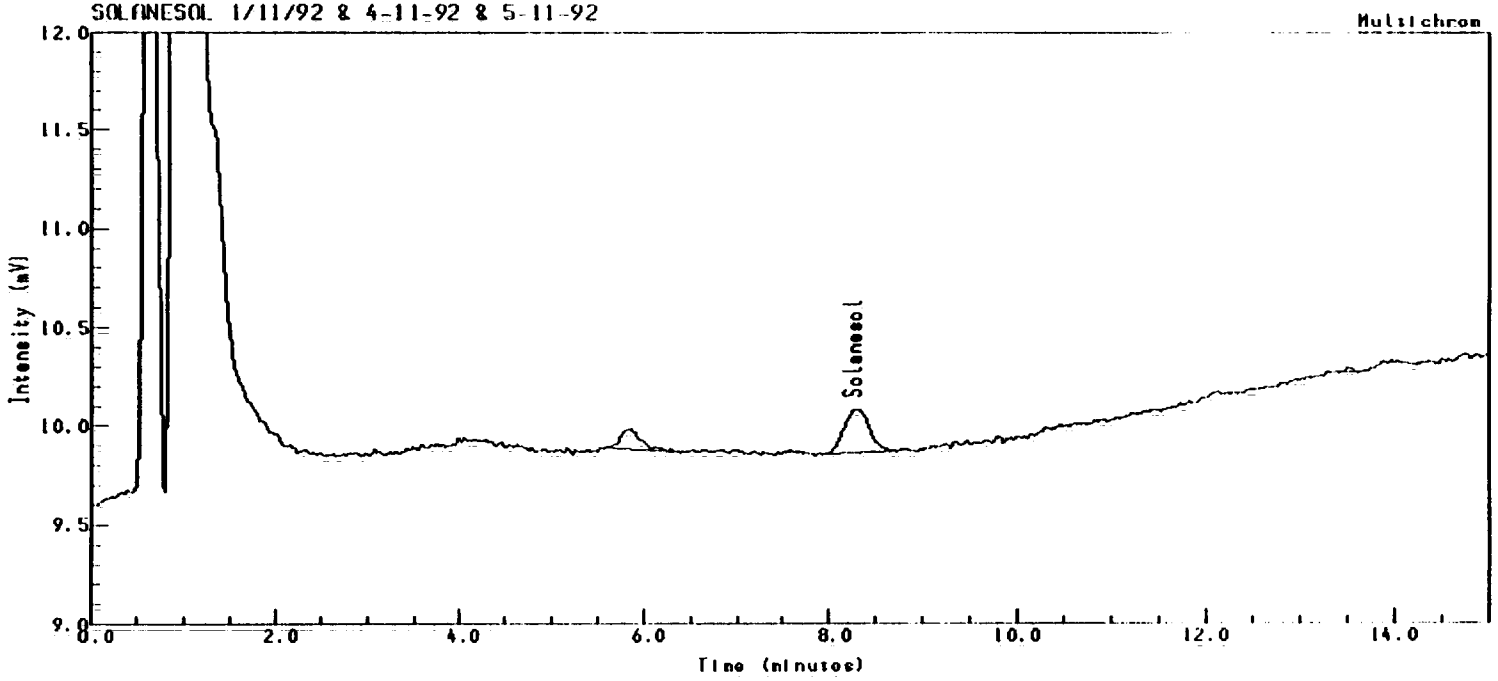
98523523

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration chromatogram
(0.01 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11.5.1.
Cal 0.010 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 8-NOV-1992 at 19.34
Reported on 3-DEC-1992 at 13.16

Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

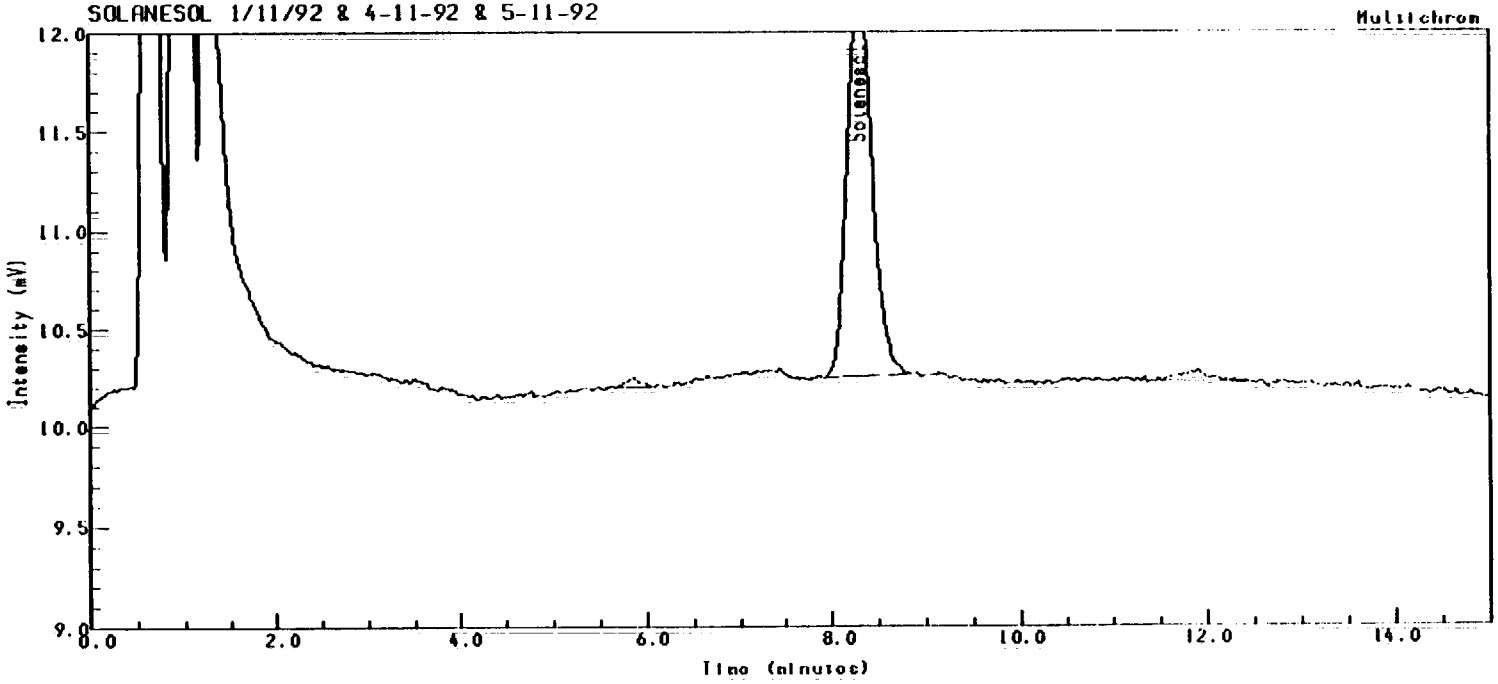
2023225287

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration chromatogram (0.10 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11,11,1.
Cal 0.100 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 8-NOV-1992 at 21:27
Reported on 3-DEC-1992 at 13:17

Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

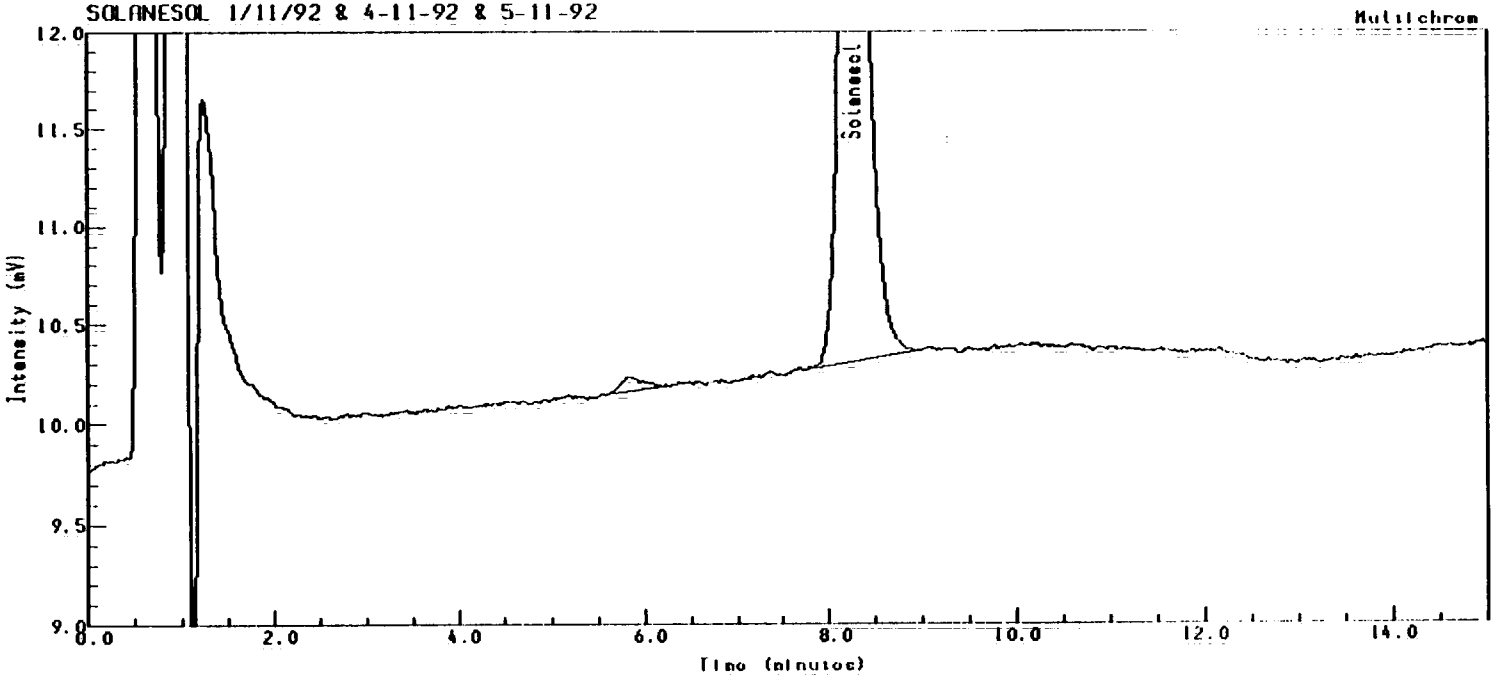
88522522802

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical standard calibration chromatogram
(0.25 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 4 S1-11,14,1.
Cal 0.250 Amount : 1.000
SOLANESOL 1/11/92 & 4-11-92 & 5-11-92



Instrument :
Channel Title : Channel #4
Lims ID :
Acquired on 8-NOV-1992 at 22:24
Reported on 3-DEC-1992 at 13:18

Method : S1-11
Calibration : S1-11
Run Sequence : S1-11

68522523202

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

DETERMINATION OF NICOTINE

PRINCIPLE

Nicotine collected on the front filter (Millipore, Teflon) is extracted into methanol. An aliquot of this extract is basified with sodium hydroxide and the nicotine is extracted into DIPE which contains triethylamine and an internal standard.

The nicotine collected on the second, acidified, pad is basified with sodium hydroxide and the nicotine is extracted into DIPE which contains triethylamine and an internal standard.

EQUIPMENT

GC, split/splitless, NPD, integrator.

Typical GC conditions are:

Column: 10 m * 0.53 mm CPSIL5 CB (Chrompack).

Carrier gas: Helium 5 mL/min.

Initial temperature: 40°C.

Hold time: 1.0 min.

Temperature programme: 15°C/min.

Final temperature: 170°C.

Final time: 2 min.

Injection volume: 1.0 μL

REAGENTS

Di-isopropyl ether (DIPE): 99.9% minimum (Romil Super Purity or equivalent)

DIPE solvent. Containing (1) 0.1 mL/litre triethylamine

(2) 2.0 mg/litre N-Ethylnornicotine.

Nicotine: Double distilled 99%+

3-Ethenylpyridine: Double distilled 98%+

N-Ethylnornicotine: Double distilled 99%+

CALIBRATION

Prepare a master solution containing 10 mg of nicotine and 10 mg 3-Ethenylpyridine in 1 litre of DIPE solvent (ie 10 μg/mL).

2023522590

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

NOTE: The DIPE solvent contains 0.2 mL/L of triethylamine and 4 mg/L of N-Ethylnornicotine internal standard. All dilutions from this master solution are made with the same solvent mixture.

Store this solution in a 1 litre volumetric flask.

This solution was prepared every week and stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with DIPE solvent as follows:

CONCENTRATION μg/mL	DILUTION OF MASTER
5.0	50 mL to 100 mL
2.0	20 mL to 100 mL
1.0	10 mL to 100 mL
0.50	5 mL to 100 mL
0.2	2 mL to 100 mL
0.10	1 mL to 100 mL
0.025	0.25 mL to 100 mL
0.00	0 mL to 100 mL

These calibration solutions cover a range of 0 to 25 $\mu g/m^3$ for nicotine and 3-Ethenylpyridine on the acidic filter. The calibration point of 0.025 μg/mL corresponds to 0.13 $\mu g/m^3$.

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

Fill an autosampler vial with each of the calibration solutions.

2023522591

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Inject each calibration solution into the GC system. Measure the area of the NPD peaks corresponding to nicotine, 3-Ethenylpyridine and the internal standard using an integrator. Prepare a calibration graph of the ratio of nicotine peak area to internal standard peak area against nicotine concentration and a corresponding graph for 3-Ethenylpyridine.

METHOD

Inject the front extract from its autosampler vial (referred to as vial A) into the GC.

Inject the acidic filter extract from its autosampler vial (referred to as vial C) into the GC.

Determine the peak areas corresponding to nicotine, 3-Ethenylpyridine and internal standard using an integrator and calculate the ratio of nicotine peak area to internal standard peak area and the equivalent ratio for 3-Ethenylpyridine.

CALCULATION

By reference to the calibration graph (or the equation of best fit), determine the nicotine and 3-Ethenylpyridine concentrations of the filter extracts in $\mu g/mL$.

Calculate the quantity of nicotine per cubic metre of air sampled as follows:

Nicotine=N*VM*1000/VA $\mu g/m^3$ for the first filter

Nicotine=N*1000/VA $\mu g/m^3$ for the second filter

Where:

N=the nicotine concentration, in $\mu g/mL$

VA=volume of air sample collected, in litres

VM=volume of methanol used to extract the millipore filter

Add the values for the first and second filters to calculate the total nicotine concentration

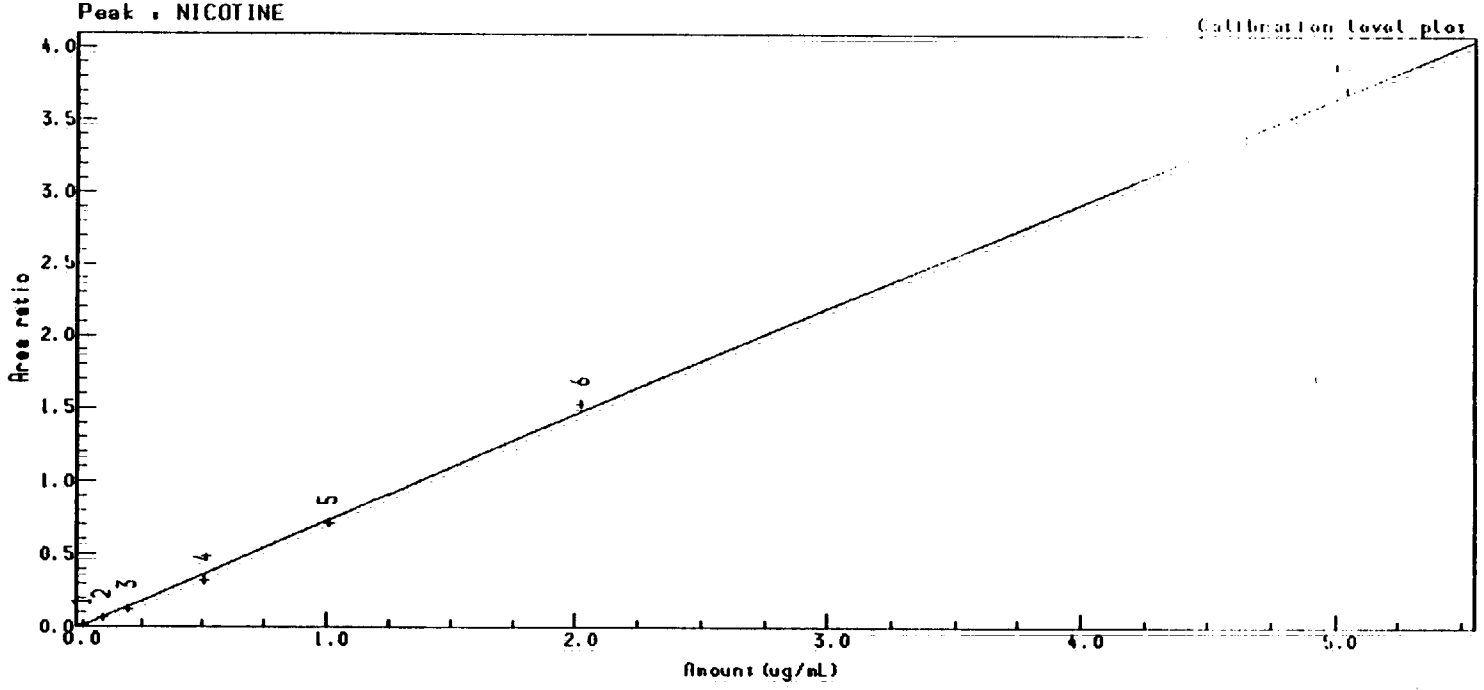
Calculate the 3-Ethenylpyridine quantity per cubic metre by the same method.

2023522592

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Calibration Name : 34 N26-11.
N26-11
Peak : NICOTINE



Constant : -0.00514
1st degree : 0.73346

Curve fit : Linear
Correlation coefficient : 0.99970
Standard error : 0.03618

Reported on 16-DEC-1992 at 14:05

SDC 16/12/92 EJ

Typical nicotine calibration plot

HUK Study no 12/64

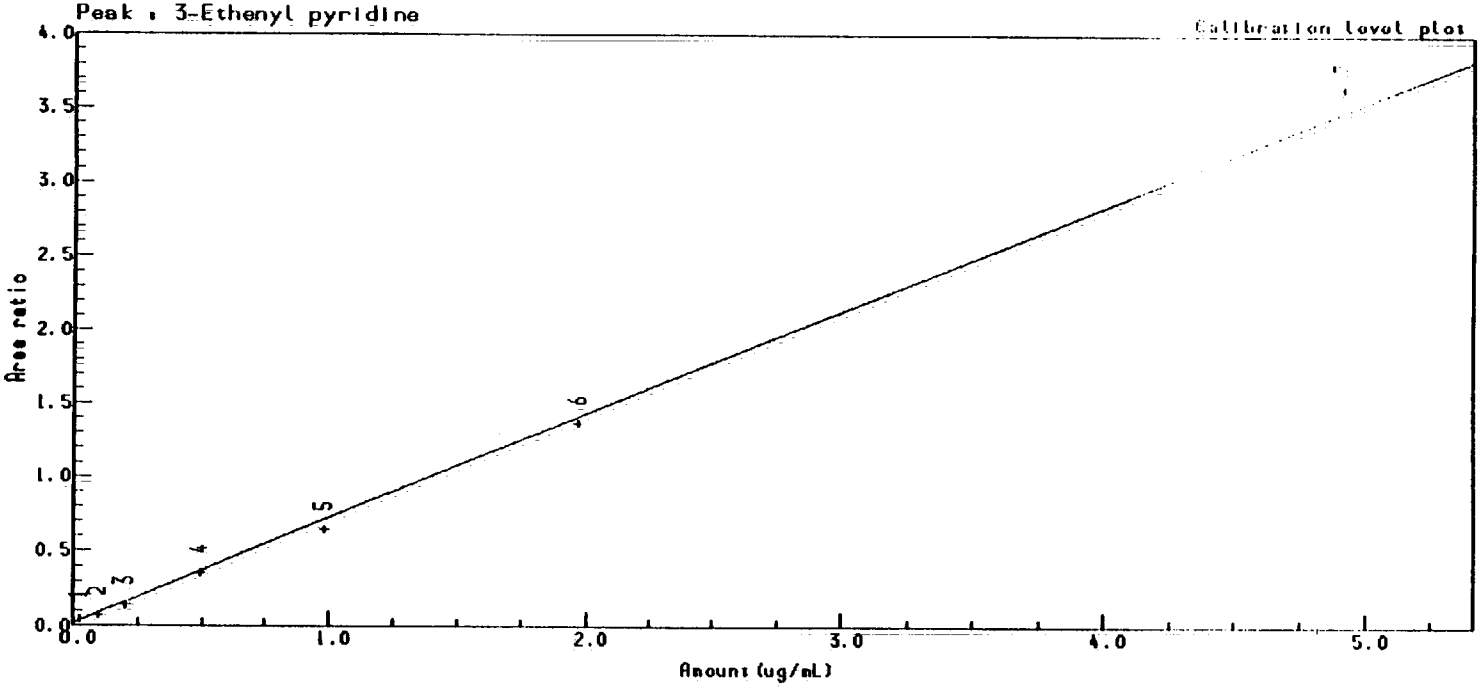
2023225293

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical 3-Ethenylpyridine calibration plot

HAZLETON Bioanalytical department Multichrom 1.8

Calibration Name : 34 N26-11.
N26-11
Peak : 3-Ethenyl pyridine



Constant : 0.02030
1st degree : 0.70461

Curve fit : Linear
Correlation coefficient : 0.99845
Standard error : 0.07948

Reported on 16-DEC-1992 at 14:04

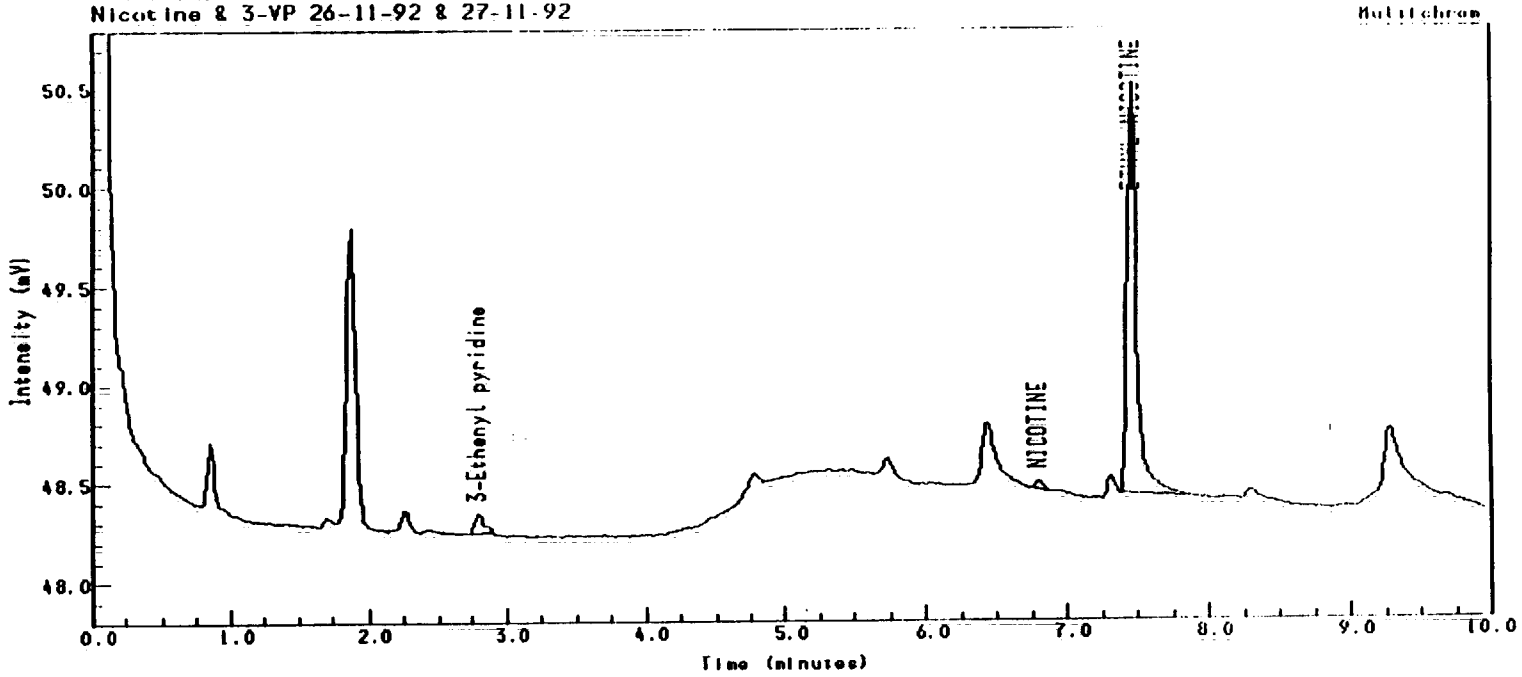
16/12/92 CJ

20235259 4

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 34 N26-11.9.1.
Cal 0.025 Amount : 1.000
Nicotine & 3-VP 26-11-92 & 27-11-92



Instrument :
Channel Title : Channel #34
Lims ID :
Acquired on 1-DEC-1992 at 11:43
Reported on 16-DEC-1992 at 14:25

Method : N26-11
Calibration : N26-11
Run Sequence : N26-11

Typical calibration chromatogram
(0.025 µg/mL)

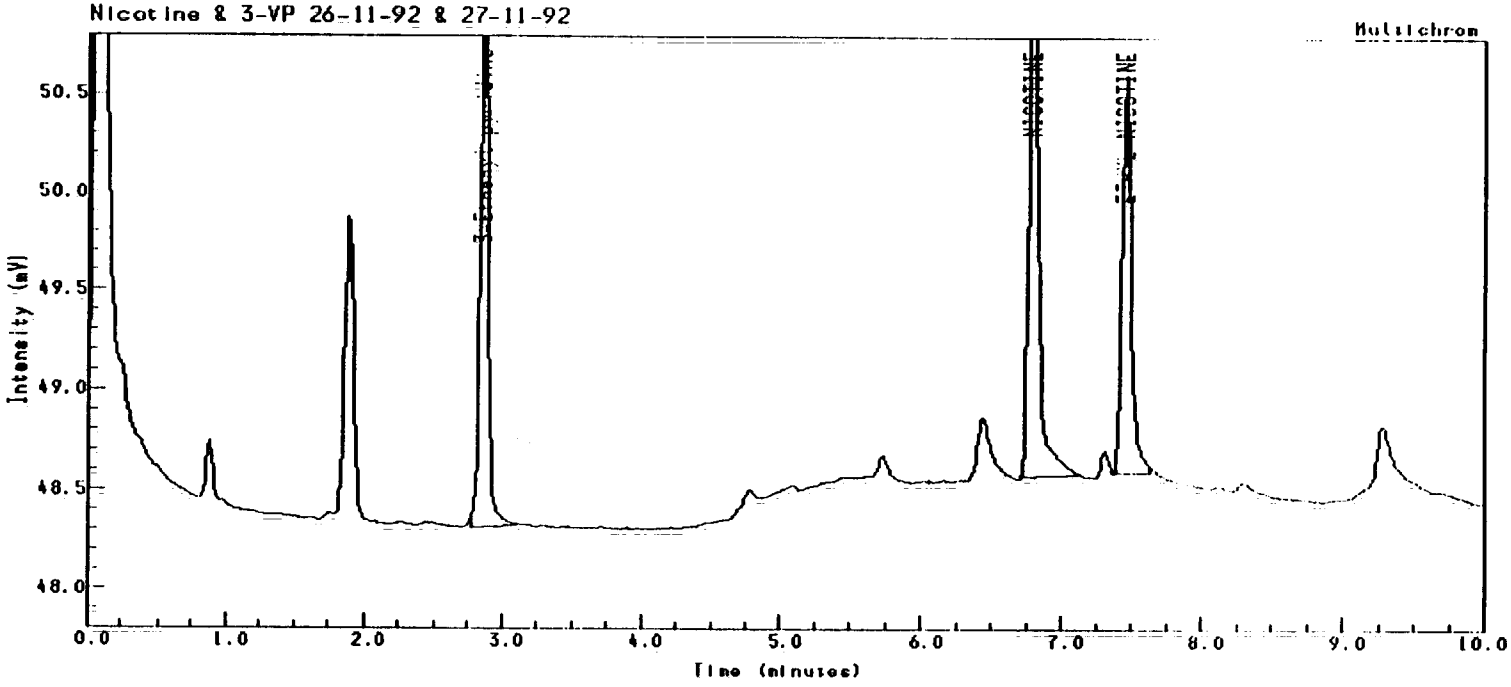
2023252595

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical calibration chromatogram
(2 µg/mL)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 34 N26-11,4,1.
Cal 2.00 Amount : 1.000
Nicotine & 3-VP 26-11-92 & 27-11-92



Instrument :
Channel Title : Channel #34
Lims ID :
Acquired on 1-DEC-1992 at 09:42
Reported on 16-DEC-1992 at 14:24

Method : N26-11
Calibration : N26-11
Run Sequence : N26-11

RJ

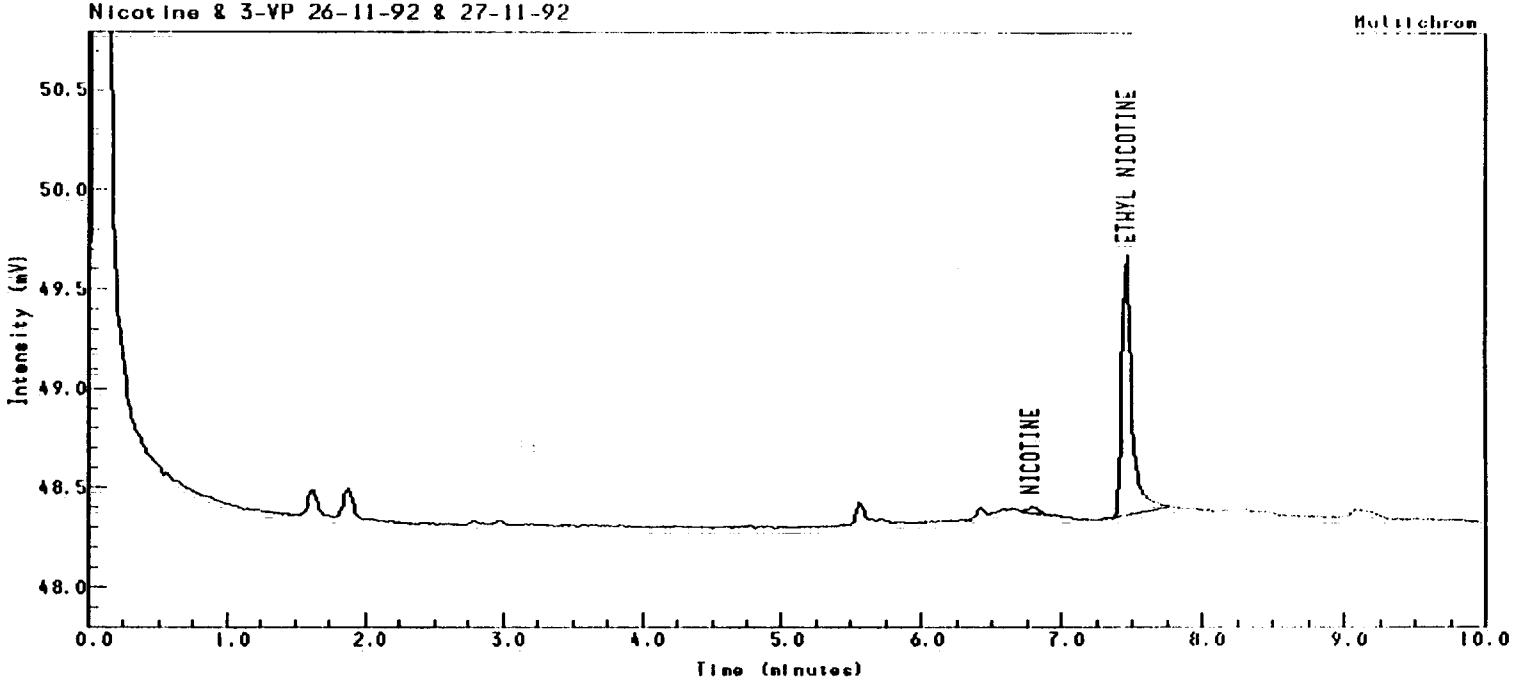
96522523202

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical sample chromatogram
(front Teflon filter)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 34 N26-11,12,1.
26-11/270-14/1 Amount : 1.000
Nicotine & 3-VP 26-11-92 & 27-11-92



Instrument :
Channel Title : Channel #34
Lims ID :
Acquired on 1-DEC-1992 at 12:56
Reported on 16-DEC-1992 at 14:25

Method : N26-11
Calibration : N26-11
Run Sequence : N26-11

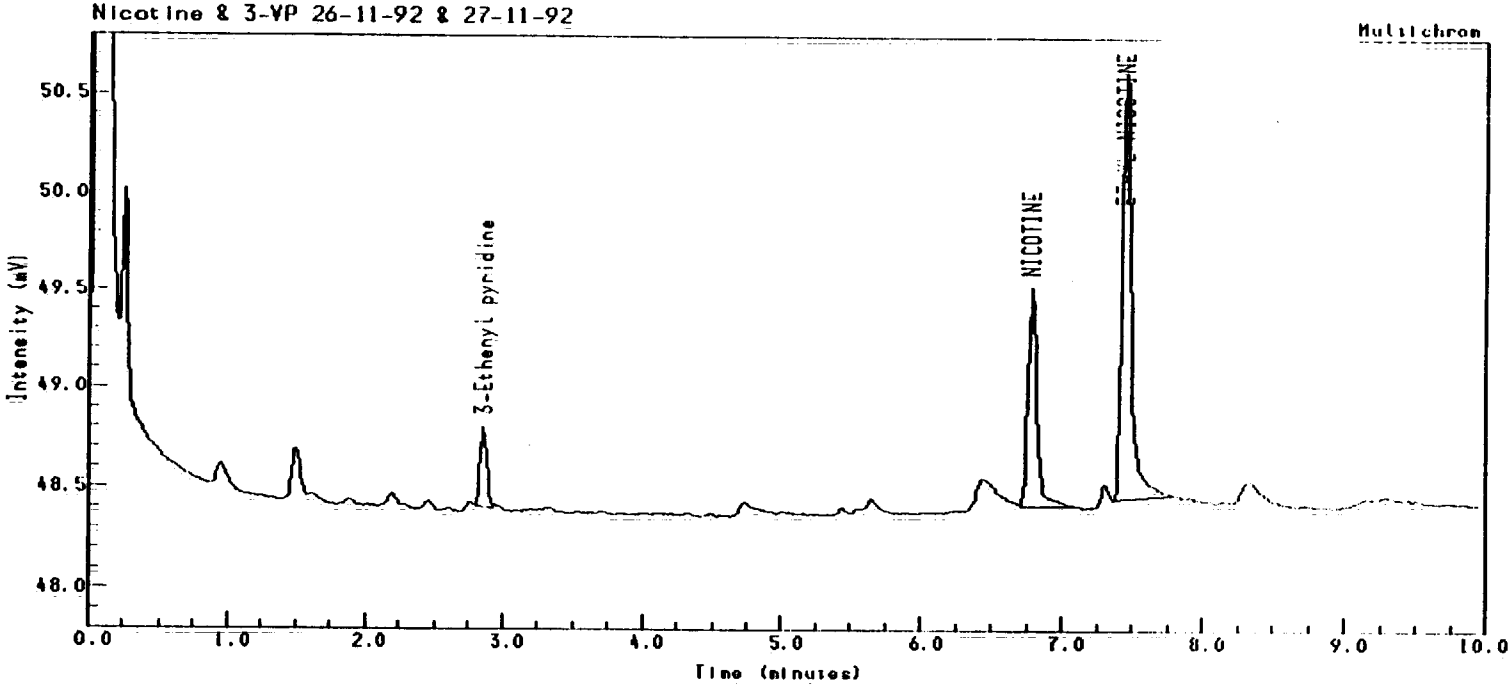
2023525597

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical sample chromatogram
(second acid filter)

HAZLETON Bioanalytical department Multichrom 1.8

Analysis Name : [JM12_64] 34 N26-11.30.1.
27-11/275-20/2 Amount : 1.000
Nicotine & 3-VP 26-11-92 & 27-11-92



Instrument :
Channel Title : Channel #34
Lims ID :
Acquired on 1-DEC-1992 at 20:12
Reported on 16-DEC-1992 at 14:29

Method : N26-11
Calibration : N26-11
Run Sequence : N26-11

ADC RV

2023225598

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

DETERMINATION OF COTININE IN SALIVA

PRINCIPLE

The pre-centrifuged salivette containing the saliva sample is removed from the freezer and thawed. Internal standard (N-Ethylnorcotinine) is added to the saliva sample. The cotinine and internal standard are extracted into dichloromethane under basic conditions. The dichloromethane is removed by evaporation under a stream of nitrogen and the sample extract is reconstituted in a known volume of dichloromethane. The cotinine is determined by capillary GC with mass selective detection.

APPARATUS

GC, splitless injector, Mass Selective Detector, Unix Chemstation data system (GC-MSD)

GC conditions are:

Column:	12 m x 0.2 mm id, 0.33 μm film thickness HP-1 from Hewlett Packard
Carrier gas:	Helium, 20 psi head pressure
Initial temperature:	50°C
Hold time:	1 min
Temperature programme:	20°C/min
Final temperature:	250°C
Cycle time:	10.33 minutes
Injection volume:	2 μL

REAGENTS

Dichloromethane:	(BDH) GC analysis grade
Ammonium hydroxide solution:	(35%) (BDH)
Ethanol:	(Hayman Labs). Absolute grade.

2023522599

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

CALIBRATION

A master stock solution of cotinine was prepared by dissolving 4.212 mg (accurately weighed) cotinine in 10 mL ethanol. Three working standards were prepared from this stock as follows:

CONCENTRATION μg/mL	DILUTION	EQUIVALENT COTININE μg/mL
5	Master (120 μL to 10 mL)	5.05
0.5	5 μg/mL working standard (1 mL to 10 mL)	0.51
0.005	5 μg/mL working standard (100 μL to 10 mL)	0.05

From these three working standards the calibration standards were prepared by dilution as follows:

CONCENTRATION ng/mL	DILUTION	EQUIVALENT COTININE ng/mL
0.5	0.05 μg/mL (100 μL to 10 mL)	0.51
1.0	0.05 μg/mL (199 μL to 10 mL)	1.01
2.5	0.5 μg/mL (49 μL to 10 mL)	2.48
5.0	0.5 μg/mL (100 μL to 10 mL)	5.05
10.0	0.5 μg/mL (199 μL to 10 mL)	10.06
25.0	5.0 μg/mL (49 μL to 10 mL)	24.76
50	5.0 μg/mL (99 μL to 10 mL)	50.03

The calibration standards are prepared in water as all saliva tends to contain a quantity of cotinine. The calibration standards are stored in a refrigerator for no longer than two months.

These calibration solutions cover a range equivalent to 0.5 to 50 ng/mL.

QUALITY CONTROL SAMPLES

Cotinine working solutions at nominal concentrations of 2 and 0.2 μg/mL are prepared in ethanol by dilution of a stock solution. These solutions are used to prepare quality

2023522600

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

control samples in saliva obtained from non-smokers at nominal target concentrations of 1, 4, 15 and 40 ng/mL. These quality control samples were prepared at the start of the study and stored in small quantities in a freezer (-20°C) for daily use.

METHOD

0.5 mL of sample/standard/QC is placed in a small test tube (12 mm x 100 mm), 50 µL internal standard solution is added, 2 mL dichloromethane and 0.5 mL Ammonium hydroxide solution. The tube is vortexed for 10 seconds and centrifuged. The aqueous layer is removed and the dichloromethane layer transferred to a second test tube (10 mm x 75 mm). The dichloromethane layer is evaporated to dryness under nitrogen. 100 µL of dichloromethane is then added to the tube. 50 µL of this is transferred to an autosampler vial and the remaining 50 µL transferred to another autosampler vial and stored at (-20°C) for backup purposes.

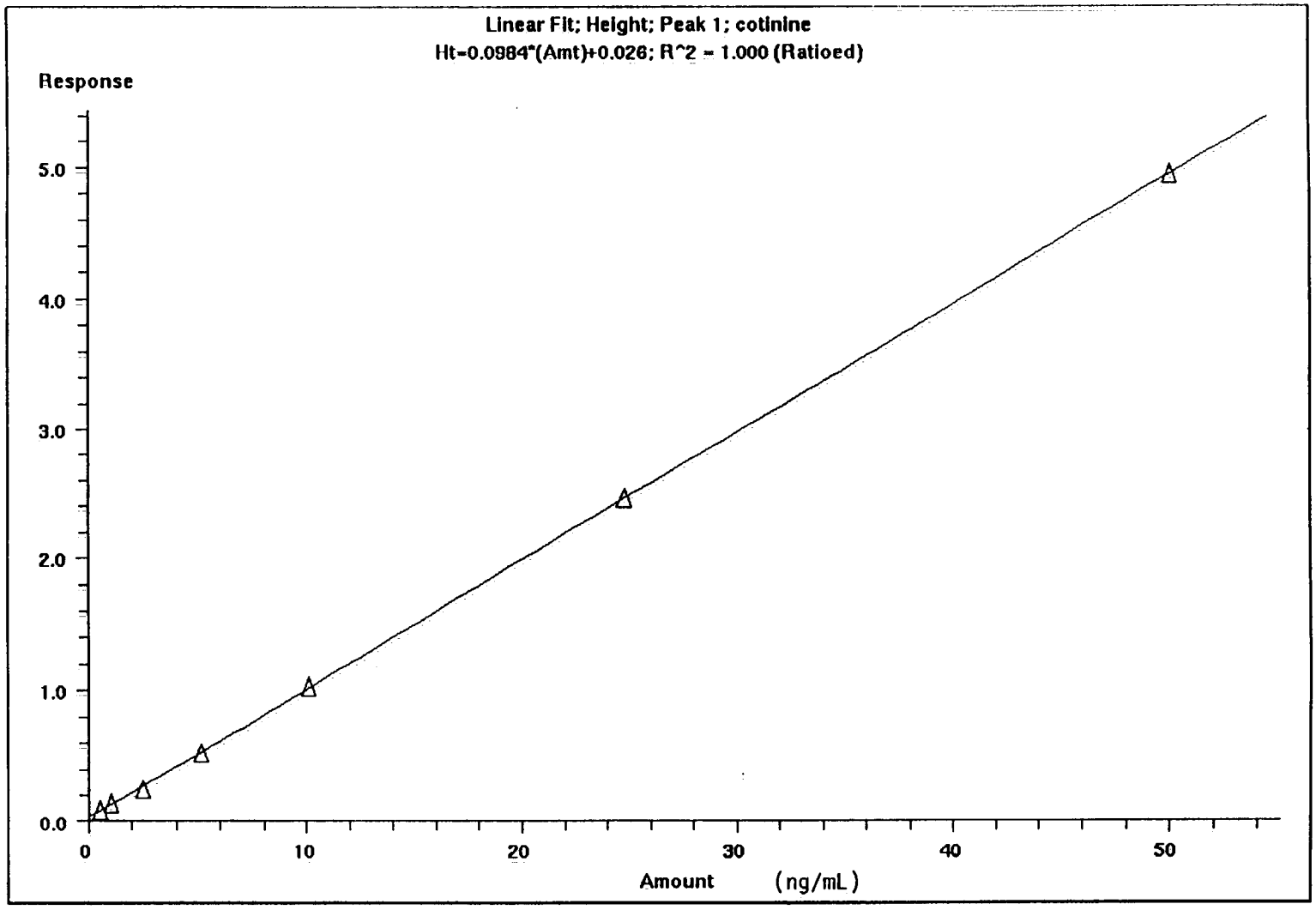
Single injections are made onto the GC-MSD. The peak height ratios of cotinine to internal standard are obtained using the data system.

CALCULATIONS

A calibration curve is constructed using the peak height ratios of the calibration standards (linear regression). The concentration of cotinine in study samples and quality control samples are obtained by interpolation of their peak height ratios.

2023522601

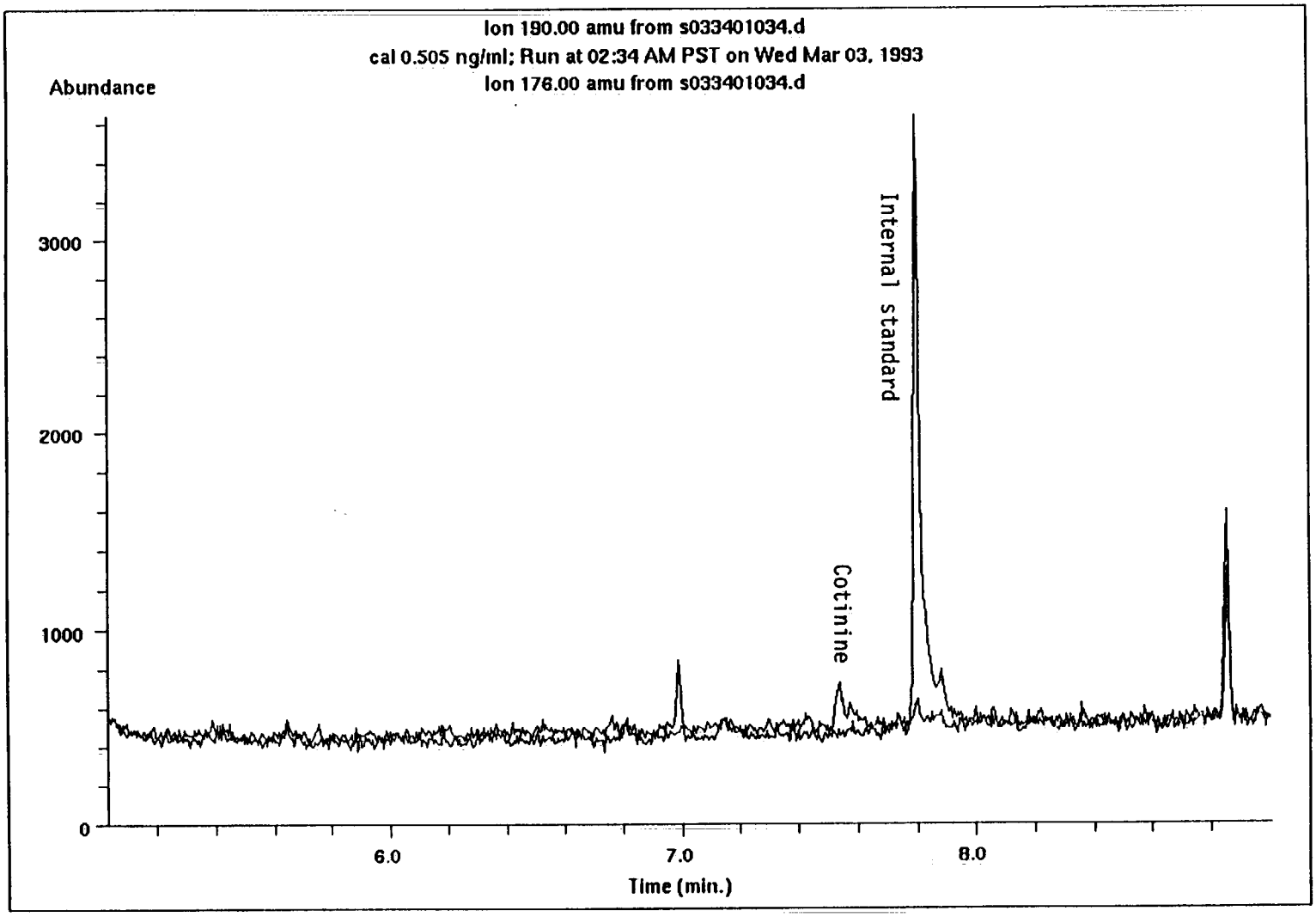
Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000



Typical cotinine calibration plot

2092252302

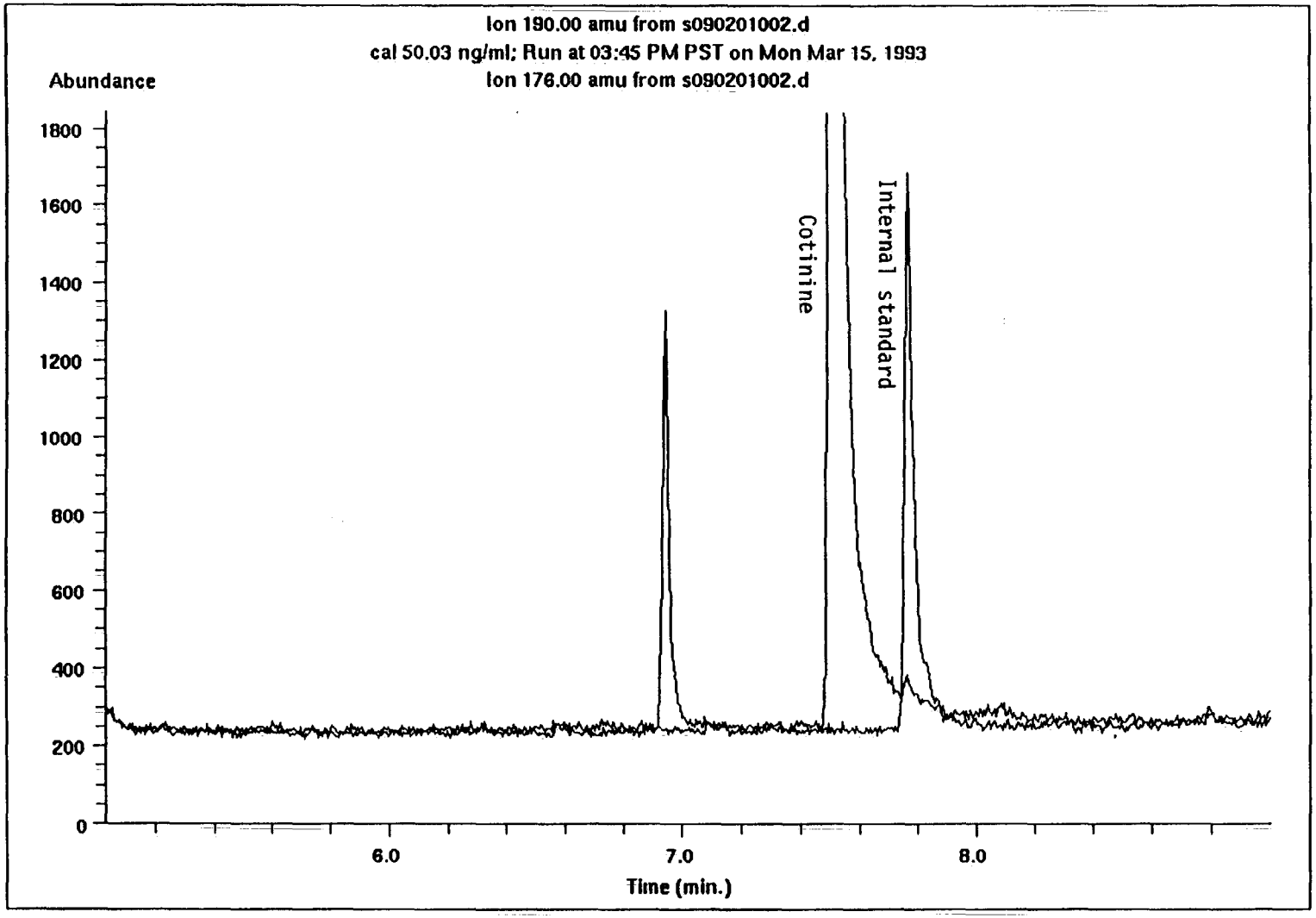
Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000



Typical cotinine calibration chromatogram
(0.51 ng/mL)

2023522603

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

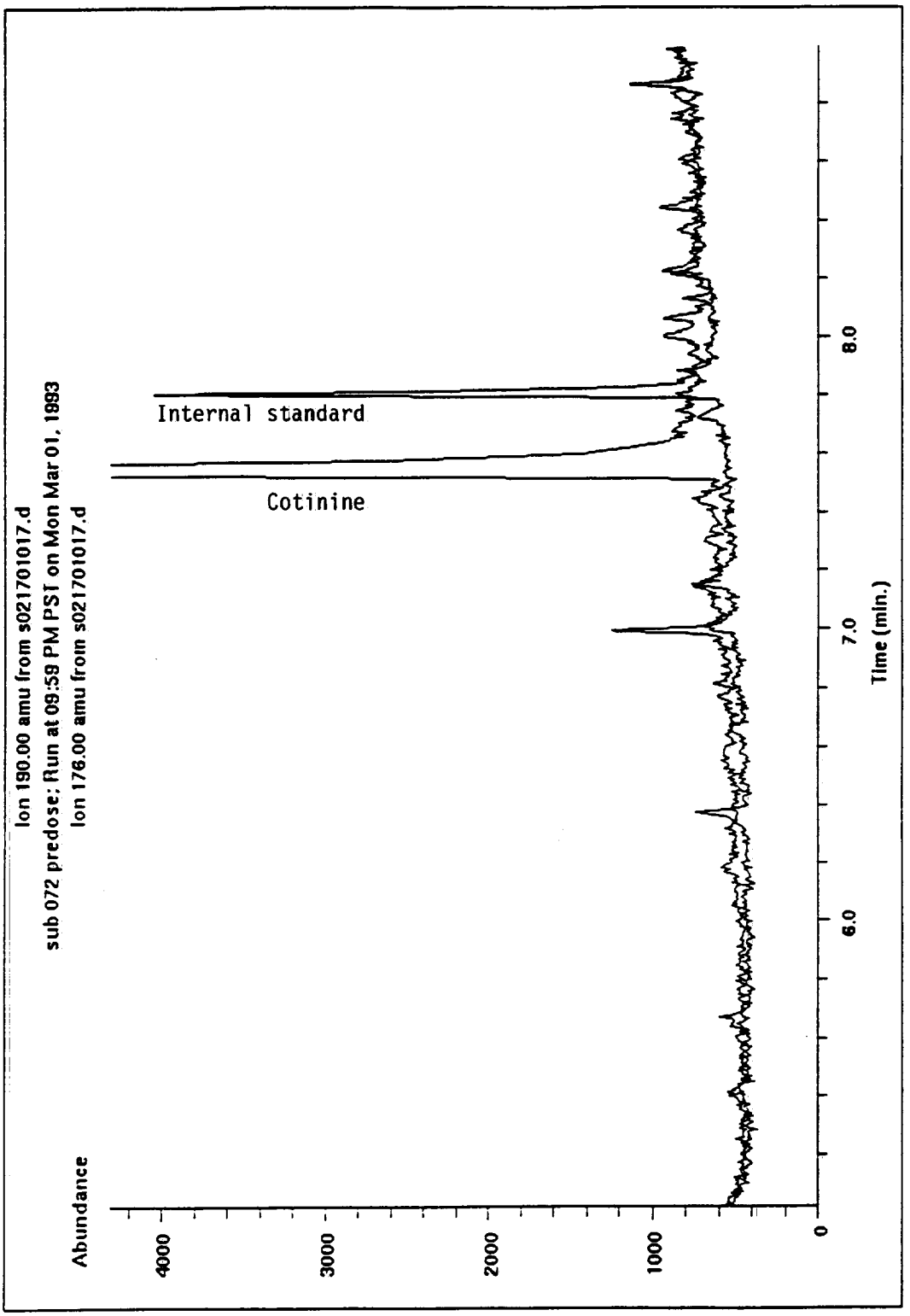


Typical cotinine calibration chromatogram
(50.03 ng/mL)

2023522604

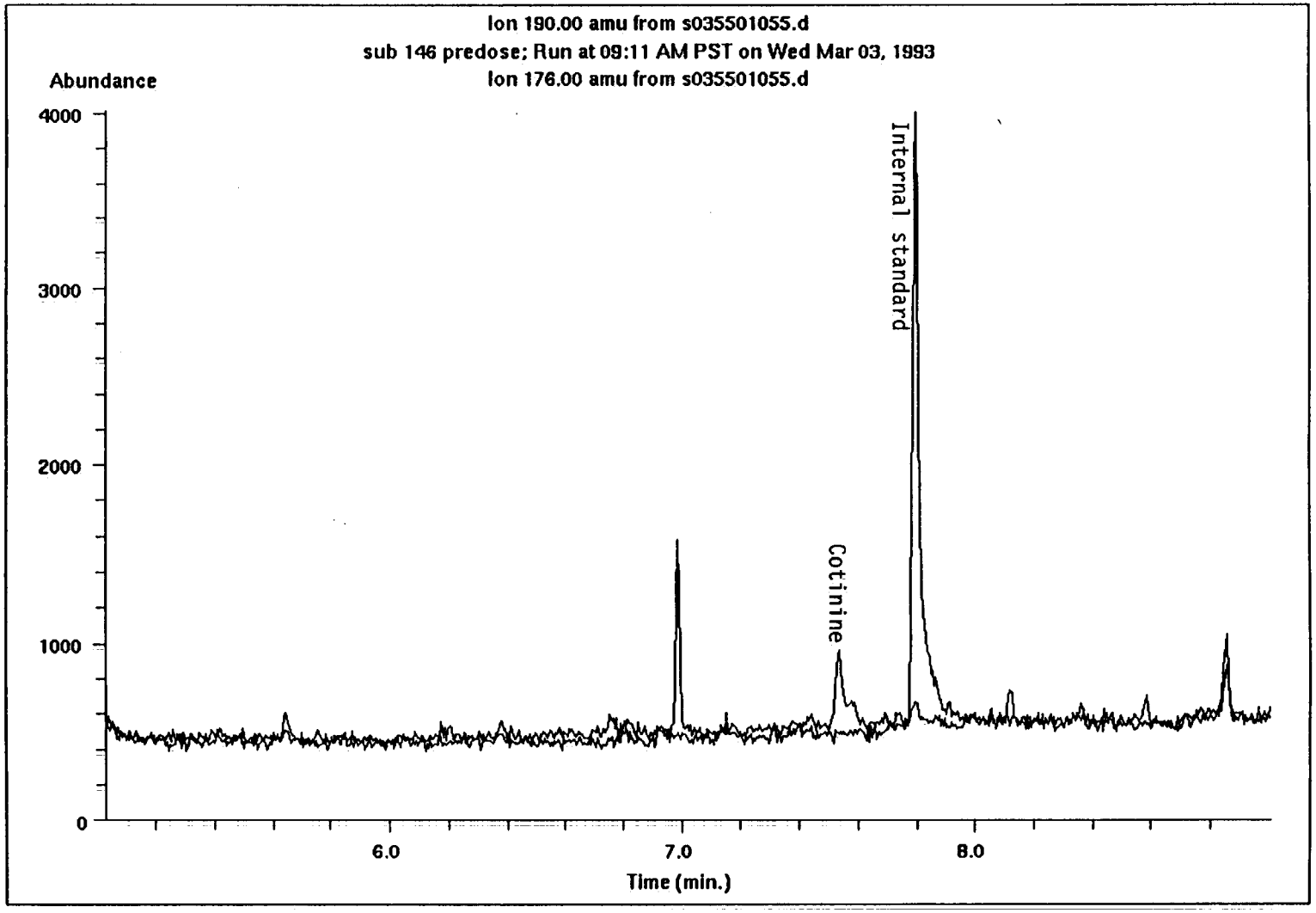
Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000

Typical cotinine sample
(high level subject ~ 100 ng/mL)



2023522605

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000



Typical cotinine sample chromatogram
(low level subject - 1 ng/mL)

2023522606

Source: https://www.industrydocuments.ucsf.edu/docs/fnlm0000